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WAR DEPARTMENT

Report 940

ACID EXTRACTION - ION EXCHANGE RECOVERY OF CINCHONA ALKALOIDS PROCESS AND PLANT DEVELOPMENT

8 June 1945

FOR TECHNICAL INFORMATION ONLY

Does not represent recommendations or conclusions of the Office, Chief of Engineers

THE ENGINEER BOARD
Corps of Engineers, U. S. Army
Fort Belvoir, Virginia

[redacted]

[redacted]

ARMY SERVICE FORCES
THE ENGINEER BOARD
FORT BELVOIR, VIRGINIA

FILE NO. 400.1 (WSS 530)

SUBJECT: Transmittal of Engineer Board "Report 940, Acid Extraction - Ion Exchange Recovery of Cinchona Alkaloids, Process and Plant Development"

TO: Chief of Engineers, U. S. Army
ATTENTION: Research and Development Division

1. Transmitted herewith is Engineer Board "Report 940, Acid Extraction - Ion Exchange Recovery of Cinchona Alkaloids, Process and Plant Development," dated 8 June 1945, which was prepared by officers of the Army Liaison Office, Foreign Economics Administration, Cinchona Research Unit, and the Technical Staff of the Engineer Board, and has been considered by members of the Engineer Board.

2. This report covers the research and development of a process and portable plant for the field extraction of cinchona alkaloids.

3. The report concludes that acid extraction of cinchona alkaloids, followed by ion exchange recovery, is an efficient process from both an economic and logistic standpoint; that the process can be carried on in a completely portable extraction plant; that acid extraction followed by alkali precipitation is a usable process where the efficiency and logistics of the method are not controlling factors; that the end product of either of the above methods constitutes a usable antimalarial; that the acid extraction - ion exchange process is directly applicable to the extraction of most of the usable alkaloids; and that the component parts of the field unit have functioned satisfactorily when used individually for other comparable purposes, but that the unit as a whole should be subjected to rigid field tests.

4. The Engineer Board concurs in the recommendations of the report, namely, that:

a. The two existing pilot plants designed and constructed at the Engineer Board be subjected to a rigorous field testing program by the officers of the Cinchona Research Unit.

b. In conjunction with this field testing, a field manual covering the extraction process and the operation and maintenance of the portable plant be written during pilot plant operation and translated into the required languages.

c. Research on ion exchange capacities now in progress at the Engineer Board be carried to [redacted]

[redacted]

5. An advance letter report on the subject development was submitted by the Board on 20 June 1945 in the interest of permitting expedited action to be taken with respect thereto prior to the submission of the complete report. The attached completed report is consistent with the advance letter report previously submitted and contains the conclusions and recommendations made therein.

FOR THE BOARD:

John W. N. Schulz

1 Incl. (in dup)
Report as above

JOHN W. N. SCHULZ
Brigadier General, U. S. Army
President

[redacted] UNCLASSIFIED

Report 940

ACID EXTRACTION - ION EXCHANGE RECOVERY OF CINCHONA ALKALOIDS PROCESS AND PLANT DEVELOPMENT

Project WSS 530

8 June 1945

Submitted to

THE ENGINEER BOARD

Fort Belvoir, Virginia

and

The Chief of Engineers

U. S. Army

Washington, D. C.

FOR OFFICIAL ACTION

by

Robert Lee Kaye
Major, Corps of Engineers

Silvio E. Ronzone
Lieutenant, Corps of Engineers

[redacted]

UNCLASSIFIED

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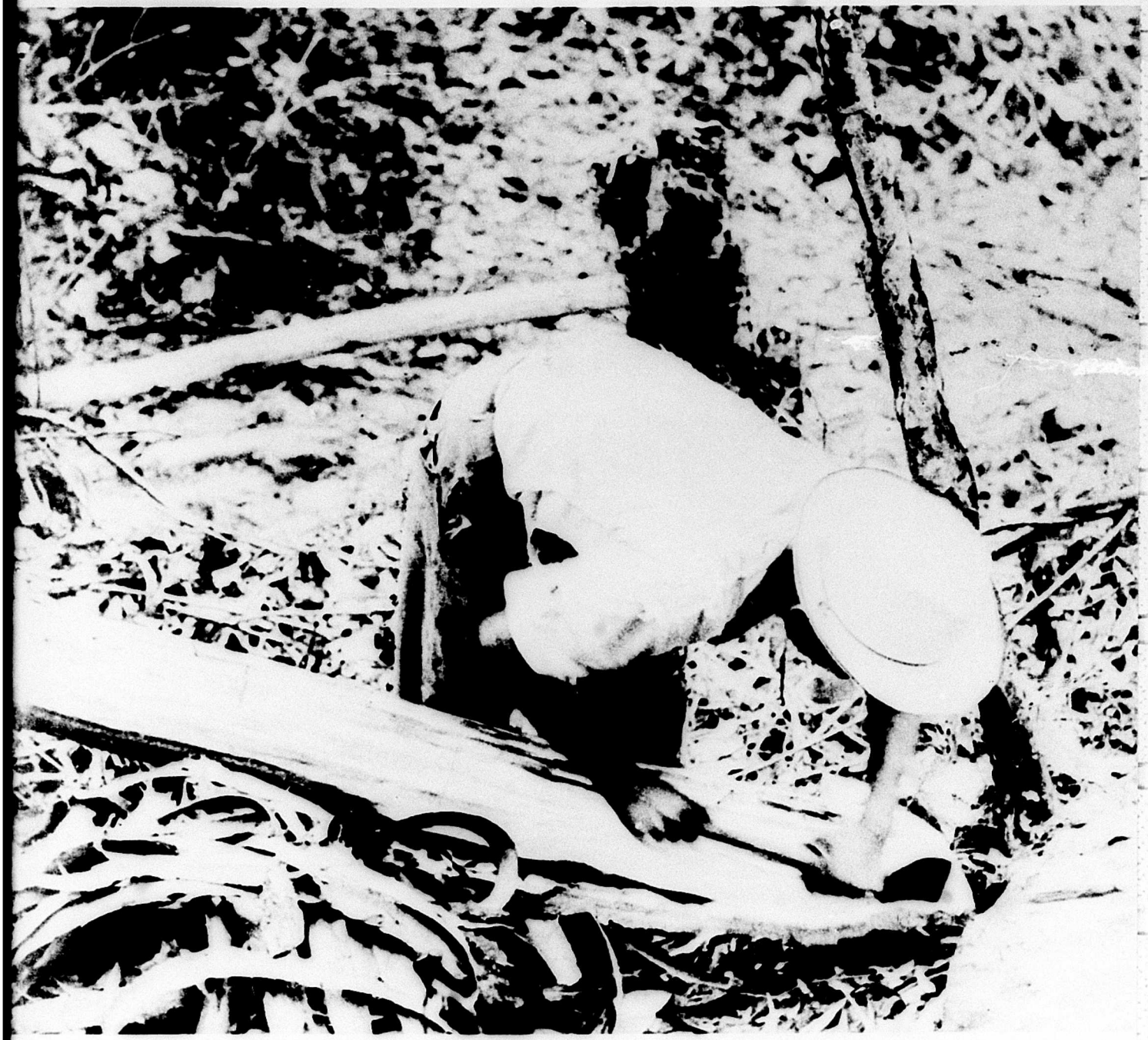


EL QUINERO

ACID EXTRACTION - ION EXCHANGE RECOVERY OF CINCHONA ALKALOIDS PROCESS AND PLANT DEVELOPMENT

I. SUBJECT

1. Subject. This report covers the research and development of an extraction process and a portable plant for the field extraction of cinchona alkaloids.

II. AUTHORITY

2. Authority. This project was initiated by letter from the Engineer Board to the Chief of Engineers dated 6 October 1944, file 400.1 (WSS 346), in which it was stated, in part, "...that facilities and technical assistance be made available for development of equipment and processes in connection with field production of quinine." The program was approved by 3rd indorsement, dated 26 October 1944, to subject letter from Army Service Forces to Chief of Engineers. The following extract therefrom authorizes the work: "Authority is granted to carry out the development program as outlined in the basic letter under Work Order No. DWS 3089."

III. INVESTIGATION

3. Personnel. The Acid Extraction Research and Development Program, under the direction of Major Robert Lee Kaye, CE, commanding officer of the Cinchona Research Unit, was carried out by Lt. Silvio E. Ronzone and a staff of laboratory assistants as follows:

Dr. H. M. Moulton,	Analyst, F. E. A.
S/Sgt. Jaspar T. Boone,	Research Asst.
S/Sgt. William M. Kerr,	Research Asst.
Sgt. Frank A. Baleri,	Research Asst.

Mr. Myron S. Mason, Engineer (Civil), Engineer Board, contributed to the design and testing of mechanical equipment and distillation technique. Mr. Norman Applezweig was employed as a special consultant on ion exchange technique. Capt. Henry Withers, CE, Engineer Board, acted as consultant on mechanical equipment, fabric tanks, filtration methods, etc. Mr. Ernest H. Sieveka, chemist, Engineer Board, carried on a special research program involving nitrogen determinations as applied to assays of alkaloid concentrates.

A. THE PROBLEM

4. General. Though the cinchona species from which quinine and its associated alkaloids are extracted is indigenous to Latin America, the Dutch, through skillful exploitation of their Java

plantations, have completely dominated the field of cinchona alkaloid production for many years. War in the Pacific cut off this source of supply to the Allies, who were forced to turn to Latin America for their antimalarial alkaloids.

a. Increased Demand for Antimalarials. The few small extraction plants operating in Latin America, plus the small amounts of bark that were being exported at the time, were wholly inadequate even so far as normal demands were concerned. At the same time, the demands for antimalarials had increased enormously due to military operations in the malarious areas of Asia, Africa, and the Pacific Islands. The resulting "slaughter harvest" of cinchona bark from the wild stands of Latin America soon exhausted all the relatively accessible supplies, and the "quineres" were forced to go farther and farther into the almost impenetrable rain forests of the Andes in search of drug-bearing bark.

b. Labor and Transportation. In these all but trackless high-altitude jungles unprecedented problems of transport and supply have been encountered. In several areas all the bark harvested has to be packed for two to three days on the backs of natives before a mule trail is reached. This has created an acute labor problem in these sparsely populated localities, since two or three men are required to pack the bark that one man can harvest. Another problem has arisen through the necessity for operating in regions where rainfall is virtually incessant. Here it is found to be impossible to dry the bark by natural means. The quality of the species found in some of these rainy areas, Cinchona pitayensis, which is valued for its high percentage of quinidine, has made it worth while to work them in spite of the fact that the bark cannot be sun dried. From the Pacific side of the western Cordillera much of the bark has to be carried out undried, thus cutting the pay load per man by about 66 percent (most green barks have a moisture content of about 66 percent); in several places dehydrators are being built at considerable cost.[1]

c. Cost. Though the Latin American Republics pioneered the cinchona industry and the use of antimalarials, the gradual decline of the industry on that continent left them unable to supply even their own antimalarial needs. At the same time, through monopolistic control, the cost of cinchona alkaloids was raised far above the level at which the average worker could afford to treat himself for the disease.

With the resumption of activity in the field of alkaloid production, these republics have become aware of the opportunity again to resume their work in the field of social betterment through the distribution of low cost antimalarial drugs to the workers in disease infested regions. In their efforts to gain control of the

1. See Appendix B, Cinchona Producing Areas of Ecuador, Colombia and Peru.

malaria problem, the Latin American republics are receiving all possible aid from the Department of State of the United States of America and from the Office of the Coordinator of Inter-American Affairs.

d. Inter-American Relations. Since it had become apparent in the earliest stages of acid extraction research that the end product could, without an elaborate refining process, be put into the form of a usable and effective antimalarial, the Office of the Coordinator of Inter-American Affairs became interested and, through the Office of the Chief of Engineers, requested that the Engineer Board assemble a field extraction unit to be used in demonstrating the process and technique of alkaloid production.

The investigation, then, has been undertaken for the dual purpose of alleviating the transportation problems of cinchona production and of providing an easy, inexpensive method of producing antimalarials for local consumption.

B. ALKALOID EXTRACTION

5. Methods of Alkaloid Extraction. Several methods of commercial extraction of cinchona alkaloids are now in use.

a. Solvent Extraction Methods. The most common method of solvent extraction is to macerate dry, finely ground bark in a ball mill or some other tumbling device with a quantity of hot solvent material, such as kerosene or diesel oil. All the alkaloids are soluble in these oils both in the form of their salts and as the natural base. With a sufficient amount of maceration, as high as 95 percent of the available alkaloids can be extracted. Recovery is made by draining the oil off the bark, which is then discarded, after which a quantity of acid solution is added to the oil and the mixture again tumbled or agitated. The alkaloids are thus converted to their salts of the acid, and go into solution in the water phase of the mixture from which they are recovered by crystalization or precipitation. Another common method is soxhlet extraction with alcohol, and recovery by means of distillation.

Neither of these methods is applicable to the use of fresh bark. In oil extraction, the oil is immiscible with water and, in consequence, the surface of the bark particles cannot be penetrated by the solvent. Alcohol, on the other hand, is completely miscible with water, so that the moisture from fresh bark dilutes it to a point where it is not an efficient solvent.

b. Acid Extraction Method. Acid extraction functions through converting the alkaloids in the bark into their salts of the acid used. In this state they are rather easily dissolved in water. Following the extraction, recovery is made by either of two methods: precipitation from solution by the addition of a strong alkali, or

adsorption directly from the acid solution by an ion exchanger. The acid extraction method is equally efficient for either dried or fresh bark, and extractions of as high as 93 percent have been attained.

Since the acid extraction method was the only one suitable for portable plant operation, it was on this basis that the preliminary work was undertaken.

C. PRELIMINARY WORK

6. Laboratory Experiments at Rutgers College of Pharmacy. The fact that the alkaloid content of wild cinchona bark varies widely creates a serious problem in the matter of procurement, since the purchaser has no ready means of making even rough tests for alkaloid content in the field. Accordingly a series of experiments was undertaken at the Rutgers College of Pharmacy in an attempt to elaborate an acceptable field method of analysis. Among other things, acid extraction was tried. Though the results obtained were not consistent enough for the purpose in mind, the fact that more than 80 percent of the total alkaloid content could be so extracted immediately suggested the use of the method for large scale work. Accordingly, a group of experiments was undertaken which prepared the background for the investigations in Latin America and at the Engineer Board. (See Appendix B.)

7. Field Experiments in Latin America. Since the Rutgers experiments were sufficient to establish the general feasibility of acid extraction it was further reasoned that the process might work with undried bark, since the nature of the solvent liquid, water solution of acid, permitted its use under these circumstances. If fresh bark could be extracted the costly and tedious task of drying would be automatically eliminated. Also it would seem feasible to eliminate the grinding process in the use of fresh bark since the fresh material retains its vascular structure more or less undamaged and hence might well be processed in the form of small "chips". On the basis of this theory the Latin America experiments were carried out. (See Appendix B.)

8. Development of the Ion Exchange Process. From the Latin America and Rutgers investigations a method of extraction by means of successive macerations in acid followed by precipitating the alkaloid from acid solution by means of a strong alkali was formulated. However, analysis of the process indicated that it was not well adapted for commercial-scale, portable plant operation, since it permits no recovery of chemicals used.

A single experiment which had been left under way at Rutgers while the Latin American research was being carried on produced results

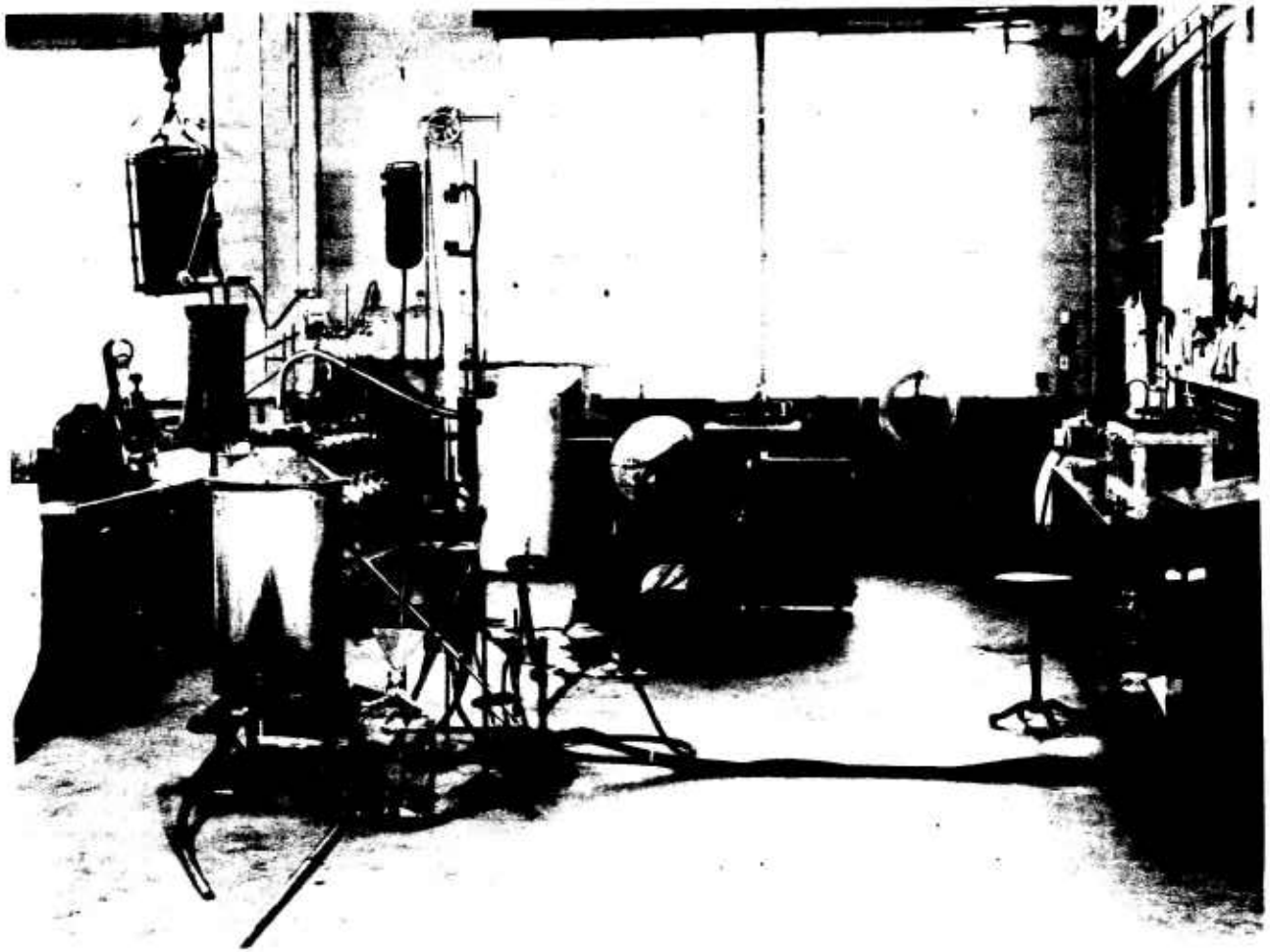


FIG. 1. RESEARCH LABORATORY, ENGINEER BOARD, FORT BELVOIR, VIRGINIA

of a very positive nature. [2] An analysis of the experiment on the basis of expanded laboratory data[3] seemed to point the way to a process more readily adaptable to field operation than the alkali precipitation method. [4] This experiment was based upon the fact that alkaloids in general behave to a marked degree like cations when presented, in acid solution to an ion exchange adsorbent. From this and succeeding experiments conducted at the Engineer Board, the process and field plant as now conceived were developed.

D. ION EXCHANGE PROCESS

9. Laboratory Development of Ion Exchange Process and Equipment. A group of experiments designated as the EB2 series was set up at the Water Purification Laboratories of the Engineer Board. The series consisted of EB2-1 to EB2-7 which were for the purpose of trying various means of setting up apparatus, regenerating and stripping the ion exchange units, circulating and stirring the solvent through the mass of bark. EB2-8A to 8C were an attempt to make three similar extractions under closely controlled conditions. EB2-8 and EB2-8D introduced variations in the matter of flow rate, stirring time and total maceration time. The EB2-8 series also furnished data when aided in determining the type of plant set-up that would be required for continuous operation. This is governed primarily by the length of time required to arrive at a suitable percentage of extraction; for since the type of operation planned makes the daily use of a predetermined amount of bark desirable, it may be seen that a 72-hour macerating time would require three macerator units, a 48-hour period, two such units and a 24-hour period, only one.

a. Maceration of the Bark. In experiments EB2-1 through EB2-8C a one-tenth scale vinyl-coated canvas tank of 25-gallon capacity was used. Later a one and one-half cubic foot filter shell of monel metal was used (Fig. 4). This was fitted with the bottom from the canvas percolator bag used in the small canvas tank, held in place by a gasket made of rubber tubing (Fig. 2). At the end of an extraction the entire mass of bark was removed from the tank, washed on a suction filter, dried, ground through a 40-mesh screen and an assay sample taken by successive quartering.

b. The Ion Exchange Reaction. The function of cation exchange in the extraction of alkaloids may be illustrated by the following hypothetical formula, wherein the alkaloid ion is represented by the symbol N, the ion exchanger by ZE, and the substituted cation by M.

$$N.Hx + HZe \rightleftharpoons N.HZe + Hx$$

---

2. See Appendix A, ZeoKarb X Adsorption Experiment.
3. See Appendix A, Preliminary Report by Norman Applezweig, Consultant.
4. See Appendix B, Review of Acid Extraction Research Program.

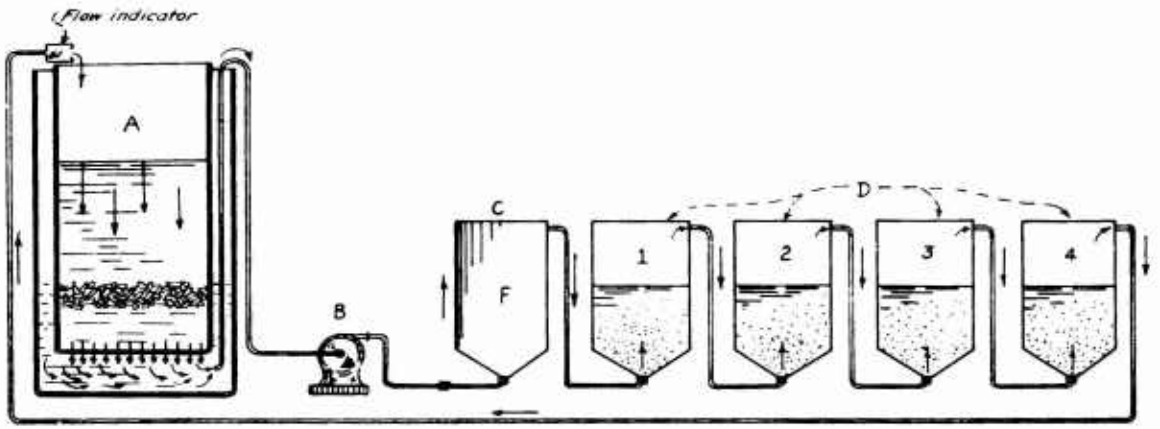
The above diagrams the function of the ion exchanger in adsorbing the alkaloid ion from the acid solution. The release of the acid molecule maintains the concentration of the solvent solution by its being pumped back into the macerator tank. Following the alkaloid adsorption phase, the alkaloid is removed from the ion exchanger by putting the system into an alkaline condition. In this state the exchanger will adsorb other cations by preference, releasing the alkaloids in the form of their natural base. The reaction in the alkaline phase may be diagrammed as follows:

$$N.HZe + MOH \rightleftharpoons MZe + N + HOH$$

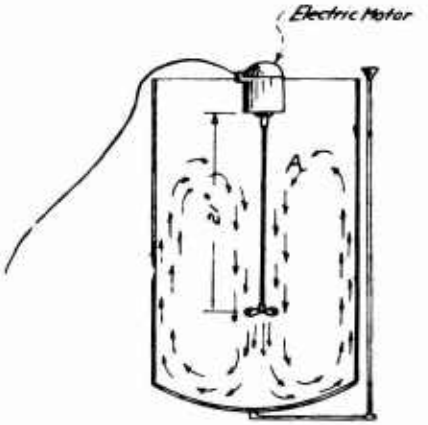
Through trial and error it has been finally established that approximately complete regeneration of the $3\frac{1}{2}$-liter ion exchange columns used in the laboratory can be obtained by passing 12.0 liters of 0.5N (Normal) hydroxide or acid through at a rate not to exceed 300 cc/min. Sodium hydroxide has proved to be the best alkali regenerant; it is widely used and therefore easily obtainable, while in effective weight it is more efficient than either NH_3 or sodium carbonate, the other two chemicals tried. In addition, both of these latter substances rule themselves out through having adverse chemical properties such as the liberation of CO_2 by sodium carbonate when brought in contact with acid.

Though several commercial acids are satisfactory as a solvent for cinchona alkaloids, sulphuric seems to rule the others out on the strength of logistics alone. Not only does it have a considerable advantage in the matter of effective weight, but it can also be transported in steel containers when in its concentrated state. Though sodium bisulphate has the advantage of being a solid, its use is not feasible because of its high equivalent weight and because of the fact that its sodium component increases the difficulties of the ion exchange process.

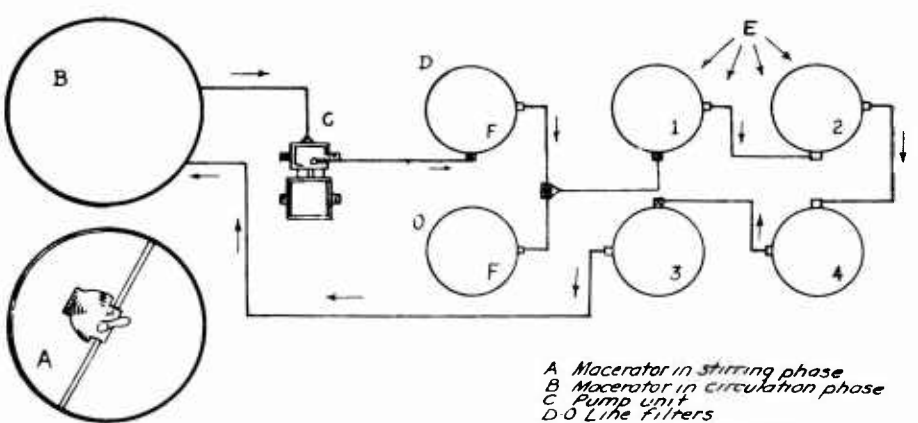
c. Circulation of Alkaloid Solutions. In view of the nature of the operation to be undertaken, it was early decided that the emphasis would be placed on upflow circulation through the ion exchange units (Fig. 2). It was reasoned that in upflow operation there would be but little more tendency for channeling to take place than in downward circulation. However, there would be no opportunity for bark particles passing through the pump to form a cake on top of the ion exchange material with resulting back pressure. Downflow operation, tried during experiment EB2-7, was found to be feasible except for the very definite tendency toward formation of a cake on top of the ion exchanger. During operation of the scale model, flow rates were determined largely by the nature of the equipment, and it was found that a maceration which in its initial stages produced a concentration that limited the flow rate to 500 cc/min or less might, at the end of the extraction, permit rates as high as 3000 cc/min. In general,



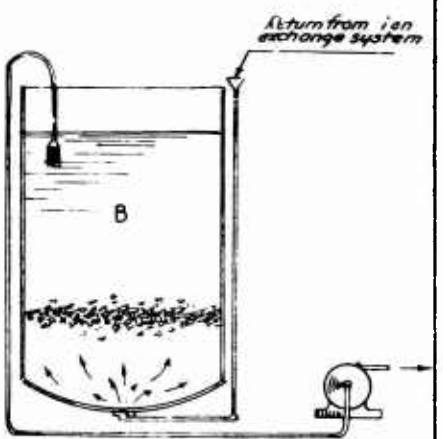
A- MACERATOR ASSEMBLED FOR DOWN FLOW, THROUGH BARK CHIPS
B- Pump
C- Line Filter
D- Ion exchange system



A- Macerator assembled for stirring phase



A Macerator in stirring phase
B Macerator in circulation phase
C Pump unit
D.D Line filters
E Ion exchange system



B- Macerator assembled for upflow through finely ground bark.

FIG. 2. FLOW DIAGRAMS, ION EXCHANGE SYSTEM

the flow rate obtainable increases in inverse proportion to the concentration of the ion being removed.* The thoroughness with which the circulated solution comes in contact with the ion exchanger in a given time depends largely on the height, diameter, and bottom construction of the container. In upflow operation the maximum flow rate, as in the case of the scale model, may be governed by the point at which bed material begins to pass through the top outlet, rather than by the speed of the exchange reaction.

d. Stripping the Ion Exchanger. At the end of an extraction, or when an ion exchange bed has become loaded to a point where no observable reduction in alkaloid content appears in a solution being passed through it, the bed must be regenerated and stripped. The regeneration is done according to the method illustrated in paragraph 9a, above. Having released the alkaloids in the form of their natural base, it remains to strip them from the column. Time requirements and general efficiency of operation make it desirable to strip the beds without drying them out. This requires a solvent that is completely miscible with water and in which the cinchona alkaloids are soluble. Alcohol is the most readily available solvent possessing these qualities. Commercial ethyl alcohol of 180° proof was used for all experimental extractions except EB2-8 and 8D, where methyl alcohol was used. It was found to be very difficult with the apparatus at hand to bring the proof of methyl alcohol back to a point higher than 140° and, considering its other undesirable qualities, such as the toxicity of the fumes, its use was abandoned. Stripping of the columns is accomplished by first introducing upflow to the drained ion exchange bed enough alcohol to fill the void spaces of the ion exchange medium; this amount serves to remove most of the remaining water from the surface of the particles. Following this, enough alcohol to fill the column, plus a small excess to provide a reserve for the pump, is introduced. This quantity is then circulated for two hours upflow, finally being drained off downflow. Another quantity, again equal to the bed capacity, is introduced upflow and, after being displaced by an upflow of water, is removed at the top of the bed. Following this procedure the beds are washed for ten minutes by an upflow of water at a rapid rate to remove any remaining alcohol, small particles of bark, and further to reduce the pH of the bed in preparation for acid regeneration.

e. Concentration and Distillation. Removal of the mixed alkaloids from alcohol solution was accomplished by means of distillation. Several types of concentrator stills, all operating on the same basic principles, were fabricated and tested. In principle, the still consists of an evaporator unit immersed in a water bath and provided with a device which distributes an even flow of the solution

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* F. C. Nachod and W. Wood: "The Reaction Velocity of Ion Exchange," Jour. Amer. Chem. Soc., LXVI (1944), 1380.

to be concentrated over the interior surface of the evaporator unit. Evaporation takes place as the fluid passes over this surface, the flow rate being so regulated as to bring the concentrate to the bottom of the unit in a fluid condition. The bottom of the concentrator is fitted with an outlet through which the concentrate can pass to the outside. The top is fitted with a steam collector dome through which the alcohol vapor is conducted to the condenser worm. Variations in the design were limited mainly to the shape of the evaporation unit and the means of distributing the flow of alcohol over its surface. It was found desirable to have an amount of water mixed with the alcohol solution sufficient to assure bringing the alkaloids and other materials to the bottom of the still in aqueous suspension. Concentrates from solutions containing little or no water were of so sticky a nature as to clog the concentrate outlet and to cause the fluid to back up in the evaporation unit. Ordinarily this is no problem, since in operation the alcohol proof cannot be held higher than 170°; however, when starting on a fresh lot of alcohol it is necessary to mix the fluid from the first and final washes with the circulated alcohol to raise the water content of the lot. Fluid concentrates from extraction of dry bark vary in color from brick red to almost black; in consistency they are generally quite sticky and contain varying quantities of suspended matter which, according to the alcohol content, may or may not precipitate upon cooling. The concentrates are always alkaline in reaction, having usually a pH of about 8 or 9. At this pH nearly all the alkaloid should be precipitated, but this is found not to occur, the material apparently being retained in colloidal solution (suspension). For this reason it seems necessary to evaporate all concentrate liquids to dryness.

The concentrating and evaporating process is complicated by the fact that temperatures above 80 C, if applied for any length of time, may result in considerable damage to the alkaloids. The use of a water bath still is the best means of temperature control consistent with field operation in jungle conditions. The fact that the proposed operations are to be carried on at altitudes as high as 8000 feet above sea level considerably lessens the danger of damage to the alkaloids through its effect in lowering the boiling temperature of water. In addition, the liquor passes through the still so rapidly that it is generally recovered at the boiling temperature of alcohol rather than at the temperature of the evaporator walls.

f. Drying Concentrates. Final evaporation of the liquid concentrates to dryness was accomplished, for laboratory purposes, in flat iron pans placed in a constant temperature oven at 70 to 75 C. An improvised forced circulation system through the oven shortened the required evaporation time by about 50%. In general, it was observed that liquid concentrate of relatively high alcoholic proof produced a hard, glazed, dark brown material upon drying. Liquid

RECTIFICATION OF CONCENTRATES

COLD PROCESS, PRECIPITATED FROM IN H_2SO_4 AT PH 9 BY NaOH, TOTAL VOLUME 3,000 CC.		
Wt. crude material	Grams	50.0115
Wt. alkaloid in crude material	"	38.1038
Total solids recovered	"	31.0396
Wt. of alkaloid recovered	"	29.3635
Wt. of alkaloid remaining in solution	"	8.7403
Percentage recovery		77.06
ASSAY DATA		
Crude Totaquine TA = 76.19 %	Refined Totaquine TA = 94.60 % ASH = 5.38 %	

COLD PROCESS, PRECIPITATED FROM IN HCl AT PH 9 BY NH_4OH, TOTAL VOLUME 3,000 CC.		
Wt. of crude material	Grams	50.0047
Wt. of alkaloid in crude material	"	38.0986
Total solids recovered	"	32.1166
Wt. of alkaloid recovered	"	29.6468
Wt. of alkaloid remaining in solution	"	8.4518
Percentage recovery		78.71
ASSAY DATA		
Crude Totaquine TA = 76.19 %	Refined Totaquine TA = 92.31 % ASH = 4.15 %	

HOT PROCESS, PRECIPITATED FROM HOT (80-90° C.) HCl AT PH 9 BY NaOH, TOTAL VOLUME 3,000 CC.		
Wt. of crude material	Grams	50.0028
Wt. of alkaloid in crude material	"	38.0971
Total solids recovered	"	26.5680
Wt. of alkaloid recovered	"	24.5435
Wt. of alkaloid remaining in solution	"	13.5536
Percentage recovery		64.42
ASSAY DATA		
Crude Totaquine TA = 76.19 %	Refined Totaquine TA = 92.38 % ASH = 8.25 %	

FIG. 3. TABULATION, RECTIFICATION OF CONCENTRATES

concentrates with a high percentage of water produced red to pink dry material of a consistency ranging from granular to crystaline.

g. Rectification of Concentrates. In general, the above concentrates will have a mixed alkaloid content of about 60%. This, considering the original bark to have had a total alkaloidal content of 5% of its dry weight, represents a concentration of 93% which, from a logistics standpoint is excellent. However, one of the aims of the process is to produce a usable antimalarial, so a further rectification is desirable.

To accomplish this purpose a simple process of dissolving in acid and selective precipitation was developed. After precipitation by means of an alkali, some of the alkaloid unavoidably remains in solution. To remove this material, which otherwise would be lost, the ion exchange process is again brought into use. The residual liquors are merely re-acidified, such dark colored materials as have been removed selectively are re-dissolved, and the whole passed through a small ion exchanger. By this means losses may be reduced to a fraction of one percent. The totaquines thus produced vary in color from white to buff and in total alkaloid content from 85% to over 95% (Fig. 3). The process may be carried on in the field or at some central concentration point, whichever proves most convenient.

10. Results of Laboratory Scale Model Experiments. The scale model plant operation involved a group of eleven experimental extractions. Experiments EB2-1 through EB2-6 were undertaken largely for the purpose of settling upon a standard operational procedure. Experiments 8 through 8D constitute the basis for establishing the efficiency and, to some extent, the logistics of the ion exchange process as applied to cinchona alkaloid extraction.[5]

In addition to determining the probable efficiency of the entire process, it was necessary to arrive at a basis for standardizing on an alkali regenerant and to find a means of using it. The subject of alcohol stripping with regard both to quantity of solvent and method of use was explored, and finally the treatment and refining of the final product was elaborated.

All extraction and recovery percentages were based on assays of original and extracted bark samples and of the concentrates produced. Various modifications of the Dutch titration method for determination of total alkaloid percentage were used throughout. Since the relative percentages of the four commercially important alkaloids would, in any case, depend on the kind and quality of the bark used, it was not considered necessary to evaluate them separately. Past experiments in acid extraction indicate

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5. See Appendix C, Data Sheets.

that no preferential extraction of the various alkaloids is to be expected.[6] A statement of the Dutch titration method, the several modifications used, and the reasons for such modification are included in Appendix C.

Tests of fluids to determine the presence of alkaloid in solution were made by means of Mayer's reagent. This is a mixture of potassium iodide and bichloride of mercury which, in the presence of most alkaloids in acid solution, forms a white to yellow precipitate. The standard method of performing the test was to introduce one drop of the reagent into one-half cubic centimeter of the fluid to be tested. In the case of alcohol, benzol, or other nonaqueous substances, a measured quantity of the fluid was evaporated to dryness, the residue shaken up in an equal quantity of dilute acid and a portion taken for testing. Alkaline aqueous solutions were made acid by adding concentrated acid dropwise until the alkalinity had been overcome.

a. Alkali Regenerants. On the theory that some damage to the alkaloids might result from the use of a strong alkali such as sodium hydroxide, attempts were made to use weaker or more easily controlled chemicals. The object in alkali regeneration is to present to the ion exchanger, while in alkaline condition, a cation which, under this condition, it will accept readily in exchange for alkaloid ions.[7] Obviously, the introduced cation has to be present in concentration sufficient to replace all the alkaloid ions to be recovered. In consequence, the hydroxyl ion concentration remaining in solution will be as great as the cation concentration retained in the ion exchanger. However, the source of danger lies in the further concentration that occurs in distillation. Here, the alcohol used in stripping the ion exchange beds is reduced to one tenth its original volume, and any hydroxyl ion concentration in alcohol solution or in the water admixture is correspondingly increased; accordingly, both sodium carbonate and ammonia were tested.

Sodium carbonate was first used in EB2-1, and immediately demonstrated the impracticability of its use by causing so great an effusion of CO_2 as to interfere seriously with operation of the column. Since it is not possible completely to saturate the ion exchange bed with alkaloid, there will always be present, at the end of an acid cycle, a certain percentage of the ion exchanger still bearing its hydrogen ion charge. As long as this situation exists, a certain amount of CO_2 will be evolved. A method of inducing the greater portion of the gas effusion to take place in an open reservoir outside the circulatory system was effective in reducing the gas accumulation within the system, but it failed to produce adequate regeneration; therefore, the use of sodium carbonate was abandoned.

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6. See Appendix B, "Acid Extraction of Cinchona Bark" by Arthur W. Walde.
7. See Appendix C, "Consultant's Report."

In some of the preliminary ion exchange experiments a combination alkali regenerant and elutriation solvent was made by dissolving anhydrous ammonia in ethyl alcohol.[8] In spite of certain apparent disadvantages the process had sufficient attractive features to warrant its further trial. The most important advantage to be realized from the use of ammonia as an alkali regenerant is that any excess concentration would be driven off in vapor form during distillation. Experiment EB2-6 used aqueous ammonia as an alkali regenerant, and resulted in so effective a demonstration of its disadvantages that, in consideration of the further difficulties presented, it was abandoned.

Experiments EB2-2 through EB2-5 were used to develop the method of regeneration by means of sodium hydroxide. An initial trial of double the calculated ion capacity of ZeoKarb passed through the column at 600 cc/min proved effective. An attempt to cut the regenerant down to the quantity actually required to fill the bed to capacity and to obtain its full use by recycling through the circulation system produced an equilibrium far short of adequate regeneration. A return to passing through the system at 600 cc/min a quantity of regenerant of twice the calculated capacity again proved effective. Since in field operation it will be necessary to regenerate the ion exchange columns separately, this procedure was made a part of routine operation in experimental extractions. As the benefit of counter current adsorption is not realized in single column regeneration, some waste of the regenerant solution was to be expected. To reduce this loss to a minimum the standard regenerant flow rate was set at less than 300 cc/min for a 4.5-liter column. Thus, the standard alkali regeneration procedure as evolved in the laboratory establishes the use of twice the calculated ion capacity of the ion exchanger passed through the exchange bed at less than 300 cc/min in .5N solution.

b. Acid Regeneration. Since logistic requirements seem to dictate the use of sulphuric acid, this same material constitutes the acid regenerant. Because the system is run in the hydrogen cycle using 0.1N acid solution, the acid regeneration is not essential; nevertheless, it is considered of value in maintaining the effective concentration in the maceration tank, and for this reason the same procedure is employed as in the case of alkali regeneration

c. Alcohol Stripping. It was originally intended that, following alkali regeneration, the ion exchange beds should be stripped completely free of their alkaloid content, as indicated by a Mayer's test performed on the effluent alcohol. To this end, a continuous slow stream of alcohol was passed through the two columns of extraction EB2-1 until 108 liters had been used. At this point the effluent alcohol evaporated to dryness, and the residue, made up to the original volume with .1N acid, still produced a definitely

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8. See Appendix A, "Preliminary Report," by Norman Applezweig.

positive Mayer's test. A somewhat different procedure was followed in experiments EB2-2 and EB2-3, which used 37½ and 20 2/3 liters per column, respectively. The total volume of alcohol for each of these extractions was applied in four separate lots. Each lot was allowed to flow through the circulation system very slowly, being collected at the outfall. At the end of each lot a Mayer's test was performed. When a Mayer's test proved the continuing presence of alkaloids, the lot of alcohol was recirculated through the system at a rapid rate for several hours. Each of these experiments persisted in showing positive through all four lots of alcohol, although in EB2-2 no more than a trace could be detected. In experiment EB2-4 no attempt was made to obtain a negative in the stripping process. Three separate lots of alcohol were circulated through each column for two to four hours and collected. Distillation of the combined first lots from the five regenerations yielded 312.81 grams of solid material. A combination of lots two and three for all five cylinders resulted in only 47.07 grams of solids. Extraction EB2-3 produced similar results, four alcohol lots having been used. Lots one and two were combined, as were lots three and four; reducing these two batches produced 96.1 and 15.79 grams, respectively.

From the above experiments it became apparent that by far the greater portion of the recoverable alkaloids were being removed from the regenerated columns in the first lot of alcohol. The average solubility in alcohol of the various alkaloids is 1 to 24; thus it can be seen that the theoretical alkaloid capacity of a 4.5-liter (820 meq/l) column could be dissolved in 3.2 liters of alcohol if all interfering factors were removed. Accordingly, the method described in paragraph 9d was formulated and used throughout the entire EB2-8 series. In view of the excellent results obtained, further laboratory trials seemed of doubtful value. Further adjustments of the stripping procedure to meet the needs of plant operation will be made in the field.

d. Model Plant Operation. Having formulated a standard operational procedure, insofar as the chemistry of the process was concerned, through experience gained from extractions EB2-1 to 7 the EB2-8 series of extractions was planned. That certain mechanical difficulties were encountered and overcome in no way detracts from the obvious significance of the final results.

From the chart (Fig. 5) it can be seen that the five experiments, EB2-8 to 8D obtained an average extraction value of 80%. It can also be seen that this average is rather heavily weighted on the low side by experiments 8A and 8B, both of which had a considerable percentage of their bark fines withheld from the extraction system. A similar computation of the average total recovery column gives a value of 73%. Removal of the two extremes raises the average to almost 76%, indicating again that the mean is weighted on

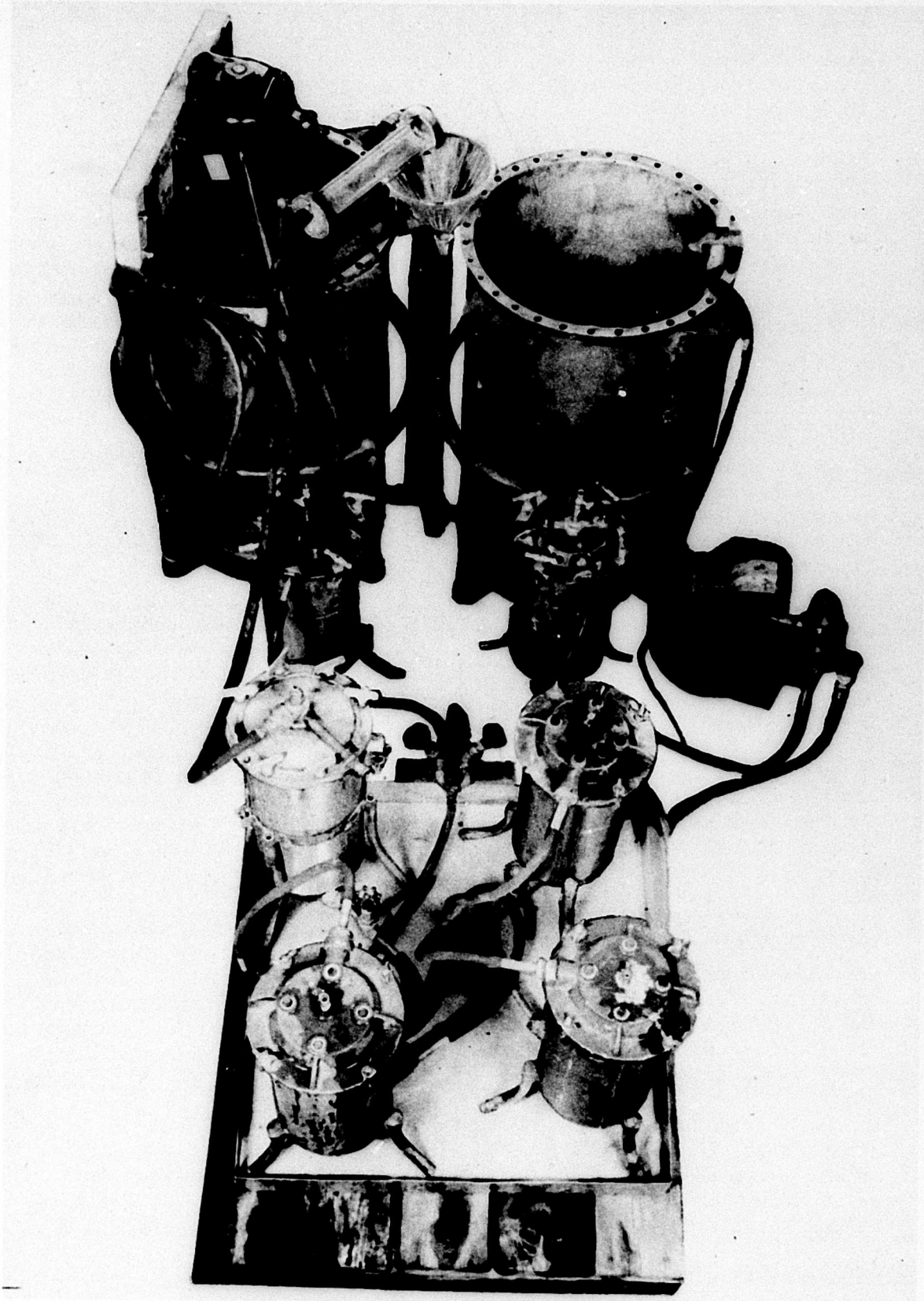

FIG. 4. SCALE MODEL EXTRACTION PLANT

TABULATION OF ACID EXTRACTION EFFICIENCY TESTS																
Exp. No.	Pounds of Bark	Orig. TA of Bark	TA of Spent Bark	% TA Extracted	Maceration Time Hrs.	Circulation Time Hrs.	Circulation Rate	No. Fluid Cycles	Stirring Time	No. Columns	Bed Vol. Liters	Total Solids Recovered	TA of Recov. Solids	% of Alkds. Extd. & Recovd.	% TA or Recovered	Remarks
6	10	4.65	1.25	72.0	5½	19½	1000	21	0	3	13.5					Recovery not attempted
8	10	2.88	0.36	88.0	72	27	1000	17.1	45½	3	13.5	116.2	41.9	45.55	40.07	Methyl alk. elutriant
8A	10	5.56		85.6*	76	20	500	8.6	8	4	18.0	300	49.7	100*	85	*Based on total recovery Fines lost in percolator
8B	10	5.56		84.4*	76	20	500	8.6	8	4	18.0	305.5	53.3	100+	75	*Based on grain size Analysis of fines lost
8C	10	5.56		90.0*	76	20	500	8.6	8	3	13.5	305.3	66.6	95	86.5	*All fines escaping percolater Recovered and assayed
80	10	3.86	0.28	93.0	100	45½	780	12.7	42⅝	3	13.5	286.0	63.1	100	100	

All regenerants and elutriant applied according to standard method as outlined.
Expt. 6 employed downflow percolation and no stirring, remainder percolated upflow with periodic stirring.

FIG. 5. TABULATION, EXTRACTION EFFICIENCY TESTS

the low side. The same is also true of the column representing the percentage recovery of alkaloids actually extracted from the bark. Here the arithmetic mean is 81.5%, with the figure similarly improved by elimination of the extremes.

From an examination of the total maceration time, stirring time and solvent cycle ratios of the various EB2-8 extractions it is apparent that a definite relations exists between this balance and the speed of extraction. Experiment EB2-8C, employing 8 hours of stirring, 76 hours total maceration and 8.6 fluid cycles, removed 90% of the total alkaloid content of the bark in that time; however, EB2-8D with 100 hours total maceration time, 42 hours of stirring and 12.7 fluid cycles produced 93%, or only 3% greater total extraction. On the other hand EB2-8 with 72 hours total maceration, 45 hours stirring, and 17 fluid cycles, resulted in a total extraction of 88%, or a drop of 2% from the standard as set by EB2-8C. It is apparent from the above that the lower limit of acceptable efficiency has not been reached in this series; however, in going back to experiment EB2-6 in which bark screenings smaller than 1/4 inch in size were used, it is found that 72% extraction was obtained in 54 1/4 hours total maceration time and 21.6 fluid cycles. It is possible to use Experiment EB2-6 in comparison with the EB2-8 group, since the bark size was the same and the columns had been regenerated by the method ultimately adopted as standard. Stirring was not employed in this extraction.

Experiments EB2-8 and 8C were very close so far as total maceration time is concerned; also, their final extraction figures are rather close. From these two experiments it appears that the increase in fluid cycles had no influence toward obtaining a greater extraction percentage. This assumption is further borne out by EB2-6, in which the time factor is reduced by 21 3/4 hours, while the number of cycles is greatly increased; here, the percentage extracted decreases sharply.

From the above it can be estimated that the lower limits for acceptable extraction place total maceration time at about 72 hours, during which 8 or 9 fluid cycles will produce the maximum obtainable extraction. The upper limit seems to have been approached by Experiment EB2-8D, in which an increase of 24 hours in maceration time produced only 3% greater extraction than the standard (EB2-8C). Since the additonal 24 hours would require the installation of another complete plant unit, the logistic values tend to rule it out.

The above values apply directly to dry bark ground through 1/4-inch mesh. With relation to operation with fresh bark

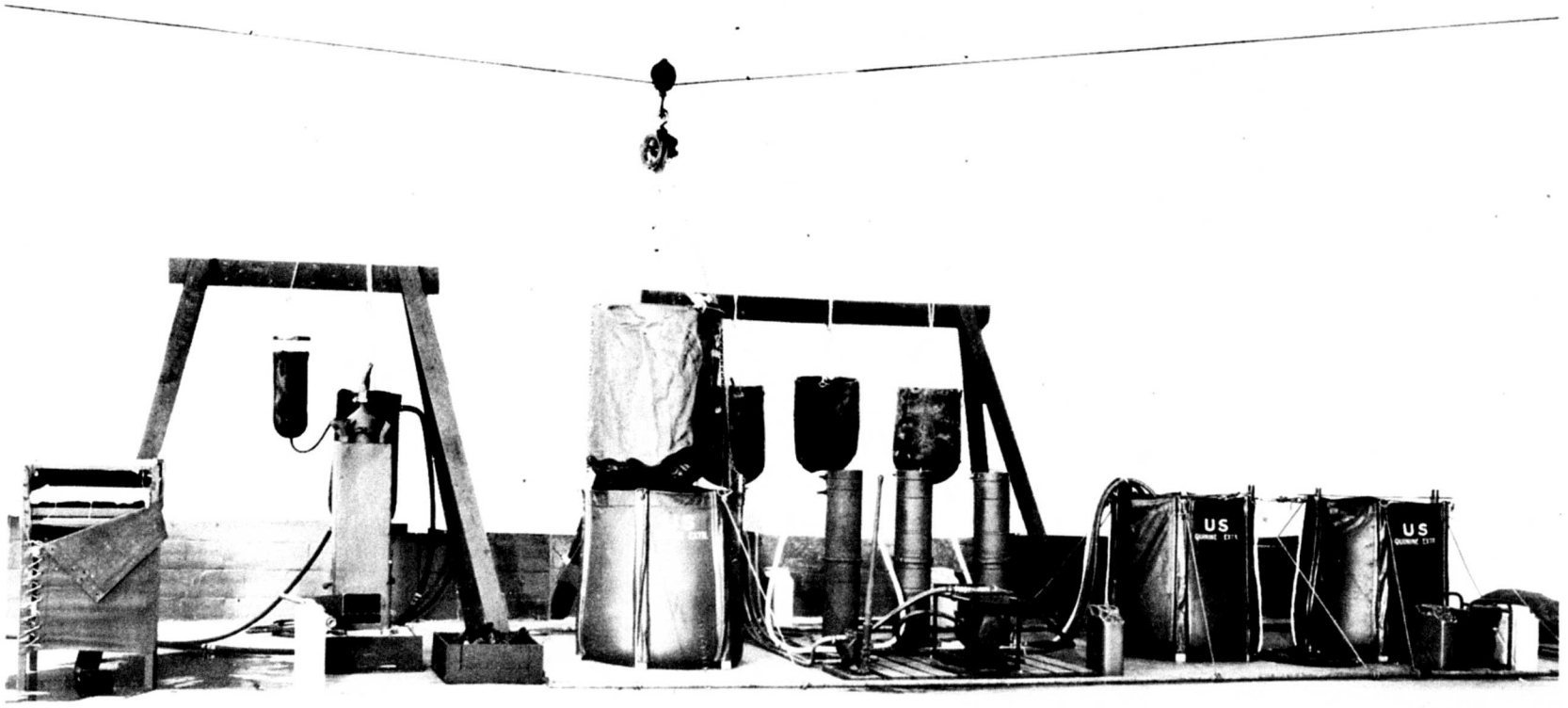

FIG. 6. FIELD EXTRACTION UNIT

they can be regarded as an approximation only. From past experience it is expected that extractions will be improved by using freshly harvested material.

E. PORTABLE EXTRACTION PLANT

11. Development of Portable Extraction Plant. The primary purpose of the research and development program was to improve the logistics of the procurement of cinchona alkaloids. The principal factor here, as in almost any field operation, is transportation. This development, then, was based upon the theory that the haulage requirements could be reduced by taking the extraction process to the cinchona forests and plantations instead of bringing the raw material to the extraction process. Thus, the emphasis in plant development was, of necessity, on complete portability (Fig. 6).

The Latin America surveys (Appendix B) established the fact that an area within walking distance of a central point could usually be so worked as to produce between 500 and 1000 pounds of fresh bark daily. It is contemplated that plant operations will not be undertaken unless at least 30 days' operating supply is available within "walking" radius. Hence, a figure of 500 pounds of bark was chosen as the basic daily operating unit, and the smallest plant component is designed for that capacity. For areas that can be more efficiently operated at a production rate greater than the minimum, several such units can be operated as a single plant.

a. Maceration Tanks. Based on the minimum unit capacity of 500 pounds of fresh bark, the maceration tank must be of sufficient capacity to hold that amount plus enough acid solution to cover the bark, and to allow an excess so that agitation of the bark mass by means of a directed stream of fluid will not be impeded.

Development of fabric water tanks by the Engineer Board furnished much applicable data on the design. A vinyl-coated fabric was selected as the most readily available chemical-resistant material suitable for pilot plant construction. While glass cloth is a more desirable base from the standpoint of chemical resistance, it was felt that sufficient data to standardize on this material was not yet available, so No. 3 cotton duck was adopted. Experience has indicated that with adequate vinyl coating on both sides this material should be sufficiently resistant to chemical action.

The most desirable shape of tank is one which furnishes the greatest height consistent with a reasonable degree of stability when erected and filled. The greater height tends to promote a more even distribution of flow through the bark, and serves to reduce the tendency toward channelling. The dimensions to give the required volume were thus established at 48 inches outside height and 40 inches inside

diameter. Six wooden staves in continuous stave pockets were provided for support, with "D" rings for guy ropes at the top of each pocket. (Fig. 8).

Preliminary tests made during the scale model operation indicated that satisfactory percolation could not be accomplished in a single tank. This is caused by the fact that the mass of bark settles to the bottom, tending to channelize the flow toward the single opening in the tank wall. In addition, it proved desirable to use some sort of sling for the purpose of placing the bark in or removing it from the maceration tank in order to avoid the necessity for pumping the acid off into another container each time a lot of bark had to be removed. For this purpose an inner container, or percolator, was designed. This percolator is a cylindrical container with vinyl-coated duck walls and a perforated bottom, its shape is similar to that of the tank, with enough difference in diameter to permit its easy insertion or removal. The top of the percolator wall is folded back and stitched so as to contain a steel ring which serves as an attachment for raising or lowering the unit (Fig. 8).

The tank-percolator unit may be operated either upflow or downflow. In downflow operation the return stream of fluid is introduced to the top of the percolator and the suction hose from the pump inserted between the tank and percolator wall. The reverse is true in the case of upflow operation, except that a strainer has to be provided on the suction hose to prevent its picking up pieces of bark. Drawings No. D-5813-1 and 2, (Figs. 29 and 30), and plans are included in Appendix D.

12. Ion Exchange Columns.

a. Bed Capacity. Based on laboratory scale ion exchange extractions, the alkaloid capacity of Zeo Karb (a carbonaceous cation exchange material manufactured by the Permutit Co.) is computed as follows: Two and one-half liters of ion exchange bed are required for every 100 grams of mixed alkaloid; thus, the alkaloid capacity per cubic foot of this particular product can be computed as

$$\frac{\text{liters/cu ft} \times 100}{2.5 \times (\text{grams/lb})} = \frac{28.3 \times 100}{2.5 \times 453.6} = 2.5 \text{ pounds of mixed}$$

alkaloids per cubic foot of bed.

Most Latin American cinchona barks have a total alkaloid content of less than 5% of their dry weight so, in using 5% as a basis for capacity computations a margin of safety is allowed. The average

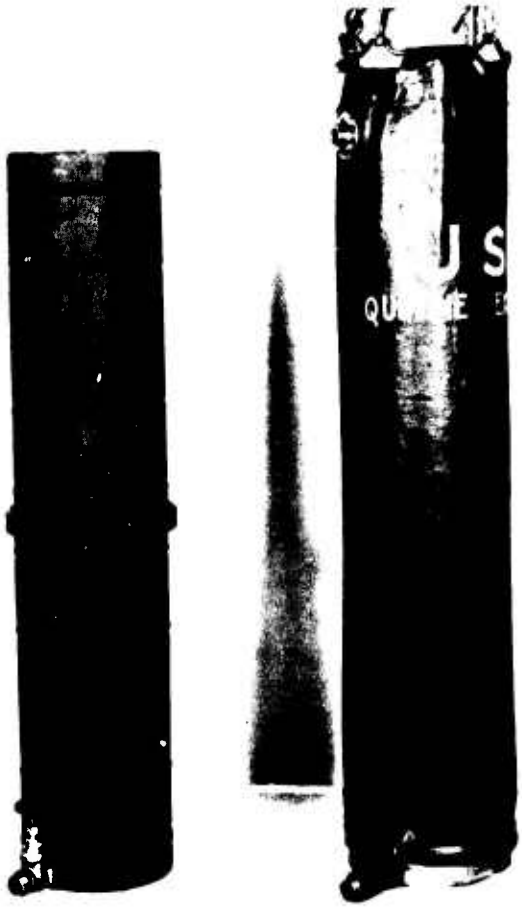

FIG. 7. ION EXCHANGE COLUMN

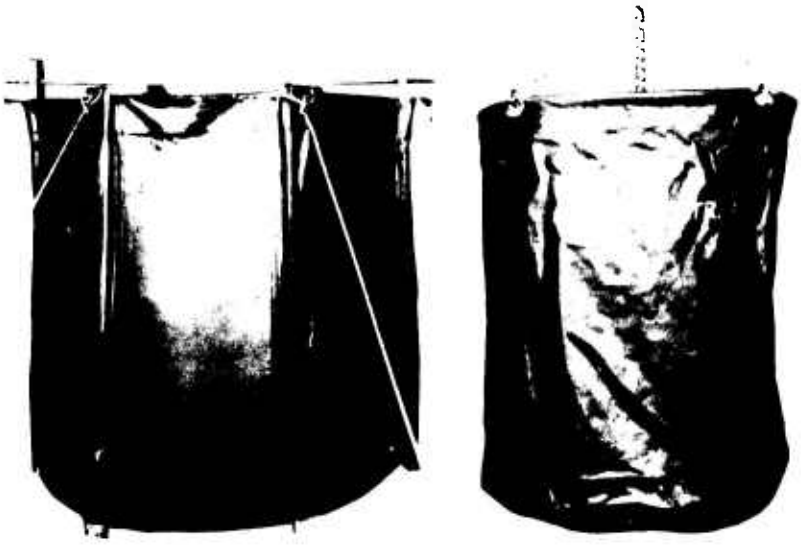

FIG. 8. TANK AND PERCOLATOR

moisture content of fresh cinchona bark is about 66%. Thus the dry weight of 500 pounds of fresh bark would be .33 x 500, or 165 pounds, at 5% total alkaloids this amount of bark would contain .05 x 165, or 8.25 pounds of mixed alkaloids. At the above calculated capacity of 2.5 pounds per cubic foot; $\frac{8.25}{2.5}$, or 3.3 cubic feet of ion exchange bed are required to retain the alkaloids from 500 pounds of fresh bark.

b. Mechanical Requirements. Manufacturers' data agree that the optimum depth of ion exchange beds is 30 inches. Though this figure is based on expected downflow, it seems to be equally well suited, on the basis of observed action, to upflow operation. It is apparent that the bed depth limit, imposed in the case of downflow by mounting back pressure which increases with the thickness of the pervious material through which the stream of liquid must pass, does not apply in the case of upflow. The bed depth limit of the upflow column is imposed by the need for portability of the apparatus. The bed expansion which accompanies upflow makes it necessary to provide at least 50% additional head room in the column. Therefore, the total volume of the container to be considered is 3.3 plus 50%, or 4.9 cubic feet.

c. Logistic Requirements. Logistic requirements prevent the use of a single unit of sufficient capacity to contain this entire amount. In addition, repeated experiments have shown that at flow rates consistent with efficient operation a "break through" of alkaloids occurs considerably in advance of the adsorption saturation point. Thorough saturation of the ion exchange medium plus complete continuous restoration of the effective acid concentration can be obtained only by counter current operation. In this method two or more columns are assembled in tandem. The first column in line receives the alkaloid solution at its maximum concentration. When a "break through" occurs in the first column, a weak concentration of alkaloids begins to enter the second column and this concentration increases as the capacity of the first diminishes. When no diminution in concentration is to be observed between the first and second columns, it is assumed that the first is saturated, and it is removed from the system for regeneration, after which it is placed back in the system in the "sweep up" position. By this means, maximum adsorptive capacity is always presented at the point of lowest ion concentration, thus assuring rapid and complete removal of the alkaloids from the acid solution.

The criteria for column design are, therefore, three columns, each of a total capacity of 2.0 cubic feet, each containing 1.2 cubic feet of ion exchange material, and each capable of easy transportation.

d. Column Design. Two distinct designs of columns were prepared, one of all metal construction, the other of a chemical and waterproof fabric with metal bottom and fittings.

The metal column design was based on the use of three cylindrical containers, each 10 inches in diameter and 48 inches overall height, with an inside available length of 42 inches, and an available volume of 1.9 cubic feet. For convenience in transportation, the column was made in two symmetrical sections, each 24 inches long and closed at the center with a clamp ring over a Victualic rubber gasket. In transportation, each section has a separate cover and may be carried full of ion exchange material, other dry chemicals, or small fittings and supplies. The lower section has a double bottom, the upper plate of which is perforated, and on opposite sides it has two 1-inch threaded-nipple hose connections between the closed bottom and the perforated plate. The top section has a single 1-inch hose connection. Both sections have a $2\frac{1}{2}$-inch threaded opening in the center of the closed end. The plug for the top opening is equipped with a $\frac{1}{4}$-inch bleeder valve.

Over the perforated inner plate, a 40-mesh non-corrosive screen to support the ion exchange material is held in place with a snap ring. The column assembled full size, may be used for either downflow or upflow circulation or, with the addition of nipples attached to the detachable lids, the separate halves may be used as half-capacity columns for downflow circulation (Fig. 7).

The alternate type of ion exchange column consists of a waterproof fabric tube, 48 inches overall length, 12 inches in diameter, open at top and bottom. A sash cord is inserted in a fold of fabric in the bottom, and a ring of 1/4-inch brass rod is similarly inserted at the top. A drum-shaped metal bottom, in the side of which is inserted a 1-inch pipe nipple, is provided with a solid plate base and perforated plate top. The fabric sleeve is forced over the upper portion of the drum and is held in place by a clamp ring against a rubber gasket, thus forming a water-tight closure. The inserted cord prevents the fabric from being pulled from under the clamp ring. Two overflow outlets, placed one above the other and fitted with pipe nipples, are provided on the side of the fabric tube, near the top of the column, which remains open (Fig. 7).

In operation, the column is loaded with 1.5 cubic feet of ion exchange medium, supported over the perforated base by a fine wire screen, a fabric disc, or a bed of sand. Flow is introduced upward through the base and overflows through the lower flanged opening at the top. Any surge of flow tending to overtop the column is diverted through the second, or higher, flanged opening. Circulation through a series of connected columns may be effected by pump, gravity flow, or both.

The advantages of this type of construction for upflow operation are (1) that flow may be regulated visibly, as necessary,

to a point of maximum velocity which does not carry over particles of exchange material, and (2) that an evenly distributed flow through the exchange mass may be visibly controlled, with hand agitation to eliminate channelling, when desired.

13. Pumping Equipment.

a. Engine-Driven Pump. As a standard component of the portable alkaloid extraction plant a gasoline-engine-driven pump was adopted for circulating the acid solution through the ion exchange system. The pump is a Rex 3M, with anodized aluminum housing and bronze impeller, directly coupled to a Lauson RSC 609 engine (Fig. 9). Pump limitations are 50 gpm or 25 psi at 3000 rpm. The engine consumes approximately 1 gallon of gasoline per 8 hours of operation. The assembly, mounted in a protecting frame for transportation, weighs 60 pounds, and without frame, 51 pounds.

In practice, the pump suction is connected by 1-inch hose to a suction strainer inserted between the tank wall and the percolator. The pump discharge is fitted with a tee and two gate valves. One leg of the tee is connected by hose to a brass nozzle fastened in side the percolator bag, and directs a strong flow upward from the bottom of the percolator. The other leg of the tee is connected by hose to the ion exchange system on either upflow or downflow circulation, in series, with return hose to the percolator.

Flow is adjusted so that the maximum flow (approximately 2.5 gpm) effecting complete adsorption of alkaloids from the acid on each cycle is established. The residual amount which the pump is capable of discharging through the nozzle serves to agitate the mass of bark in the percolator, eliminating channelling and assisting in breaking down the bark tissue.

b. Hand-Operated Pump. For use in a hand pumped circulation system through ion exchange columns, and for general auxilliary use in providing water supplies, a hand pump was selected. The pump requirements were light weight, simplicity of construction, resistance to corrosion and ease of repair. The capacity requirements were based on the energy one man could exert consistently without undue fatigue. The easiest and least tiring method of pumping has been proven by various authorities to be that of a vertical lever, pivoted at its base, with an arc of travel of about 30 inches at its upper end.*

The Red Jacket house force pump, Model 147, of 3-inch bore and stroke was selected as an ideal size, but its weight in cast iron construction was heavy, 57-59 pounds. On request of the Board a similar pump was especially constructed in the same pattern, substituting aluminum castings for iron and eliminating the air bell.

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* Ref. "American Civil Engineers' Handbook," Merriman P. 144.

The pump has a brass-line cylinder, bronze valves and seats, and brass bushings set in the casting at all friction points. It weighs 27 pounds with wood handle and 2-inch wood base, but when operated in isolated areas may be carried without handle or base, weighing 21 pounds. The pump may be spiked to a flattened log and a handle cut from a sapling (Fig. 10).

Test of a pilot model of this pump showed yields as follows:

Pressure psi	Total Strokes	Elapsed Time Minutes	Total Yield Gallons	GPM	Strokes Per Min.
0	140	3	32	10.5	47
5	140	3	31	10.3	47
10	120	3	28.5	9.5	40
15	120	3	27.0	9.0	40
20	66	2	12	6.0	33
30	80	2	15	7.5	40
40	55	2	10	5.0	27.5

c. Corrosion Resisting Metals. All aluminum in the pumps is anodized, and experiments with such material in the low acid concentrations employed in the process show little corrosion. In any event, no iron may be permitted to come in contact with the acid, since tannates are formed which greatly discolor the solution and the concentrates. Construction of brass, bronze, and aluminum is, therefore, considered expedient.

14. Hoses. For connection of tanks, pumps, and ion exchange columns into a circulation system, two types of hoses are provided. The equipment is universally provided with fittings for 1-inch rubber and fabric, wire-inserted suction hose, in 5 and 10-foot lengths, with brass screw-type fittings furnished with rubber gaskets and 1-inch straight IPS threads. Brass bushings are provided for all openings to adapt them also to 3/4-inch, straight IPS thread fittings for a 2-ply, fabric-reinforced, rubber "garden hose". This hose may be used alternately with the 1-inch hose for all purposes where maximum pumped flow and pressure are not desired, and it is particularly applicable to the lightweight, hand-operated unit. Any standard hose of this size may be used if equipped with no ferrous fittings and is usually available in all trading centers.

15. Distillation Unit. The original conception of the still was a cone, immersed point down in a water bath and heated by solid fuel. The top of the cone was closed with another cone, point up, leading to a condenser. The bottom of the cone was provided with a tube leading through the water jacket, and furnished with a closed container to receive concentrates. A perforated tubular ring around the cone at the point of greatest diameter provided an adjustable flow of alcohol solution from an elevated container.

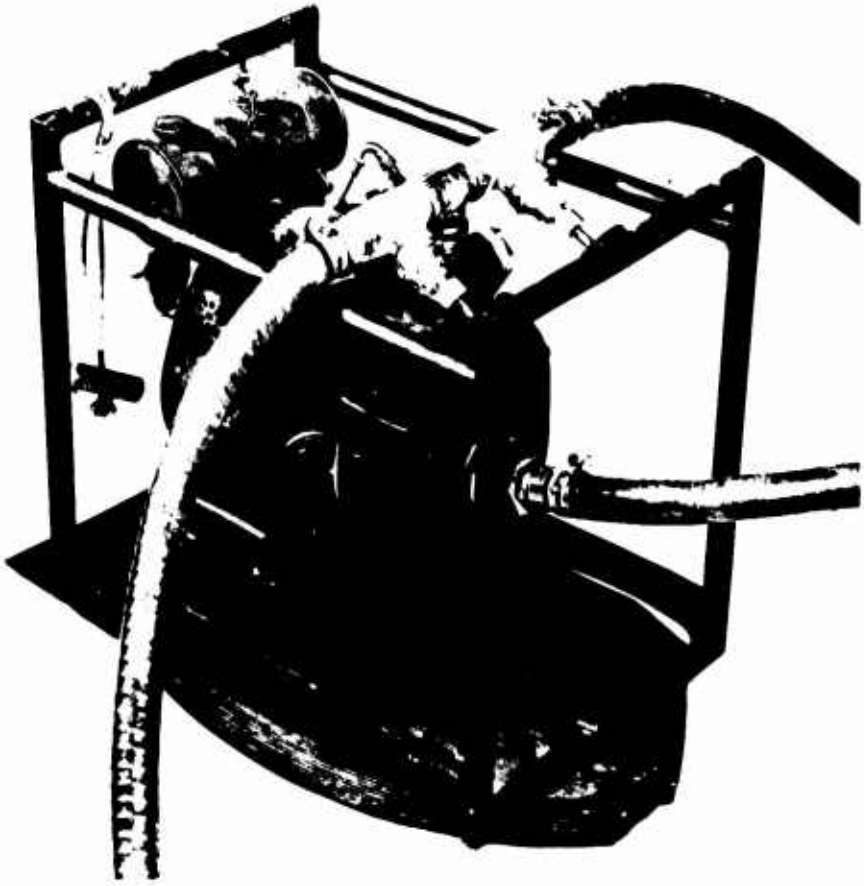

FIG. 9. ENGINE DRIVEN PUMP

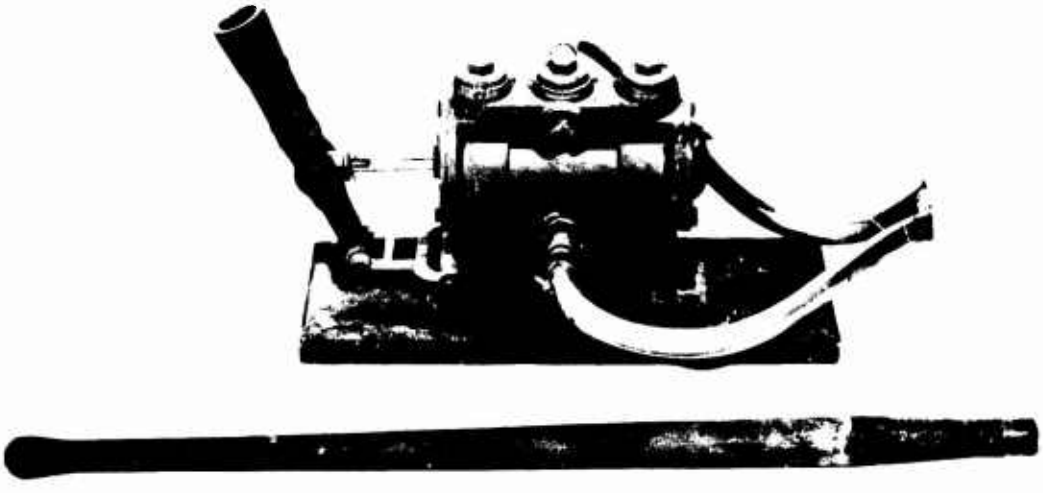

FIG. 10. HAND PUMP

PRODUCTION - EFFICIENCY DATA

EXPERIMENTAL STILL, CYLINDRICAL TYPE

Run No.	Elapsed Time	Volume of Influent (Ethyl Alc. & H_2O)	Volume of Distillate	Rate of Distillation CC/Min.	SP GR of Distillate	% Alcohol of Distillate	Vol. of Concentrate CC	SP GR of Concentrate	% Alcohol In Concentrate	Vol. Alcohol Lost In Conc.	Efficiency of Recovery %	Influent Rate GPH	
1	5'0"	2280	980	196	.863	80	1300	.950	41	500	65	27.36	Full Flow
2	5'15"	2170	1010	192	.860	81	1160	.940	47	508	66	24.78	
3	5'20"	1635	1000	182	.885	80	635	.975	42	124	89	17.70	3/4 Flow
4	5'10"	1960	1000	198	.860	81	980	.955	76	341	75	22.74	
5	5'20"	1340	1000	187	.883	80	340	.980	82	63	94	15.06	1/2 Flow
6	4'50"	1435	1000	207	.870	79	435	.980	14	29	97	18.00	
7	4'55"	1080	1000	203	.885	68	80	1.00	0	0	100	13.20	1/4 Flow
8	4'30"	1091	1000	207	.885	68	91	1.00	0	0	100	13.56	

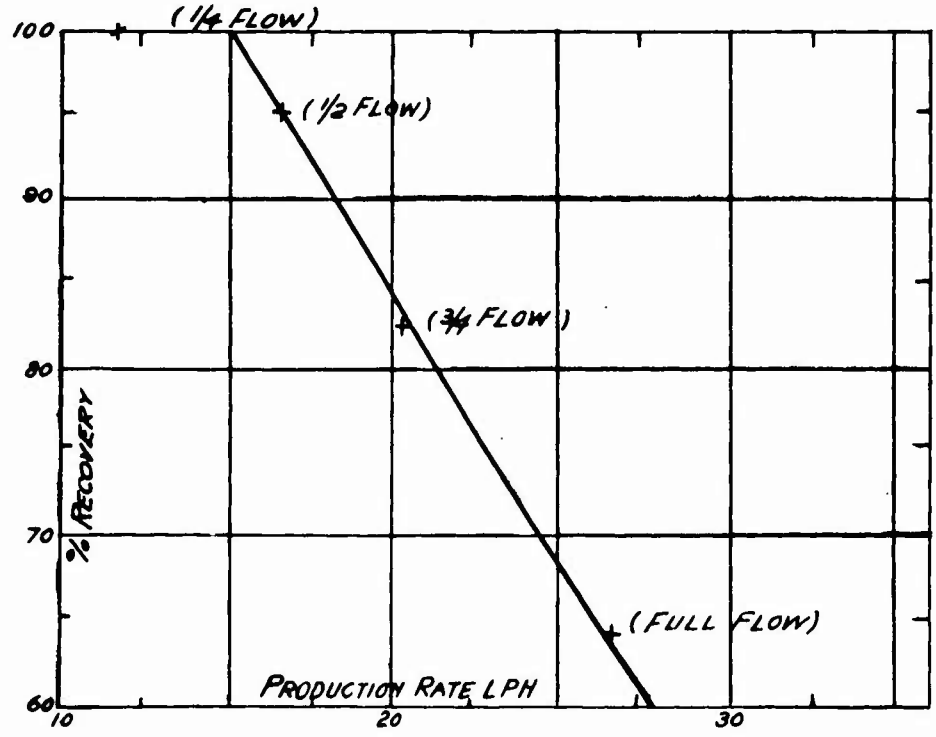

FIG. 11. PRODUCTION-EFFICIENCY DATA, EXPERIMENTAL STILL, CYLINDRICAL TYPE

A still of these general characteristics was constructed of laboratory glassware and tested. The principle of operation proved satisfactory, and a larger still was constructed with an aluminum cone, 15 inches in diameter and 18 inches in depth, heated in a water bath over a charcoal furnace. The yield of this still averaged 5 gallons per hour, and with forced draft heating was capable of producing 7 gallons per hour. Recovery of alcohol in quantity compared to the amount originally introduced averaged 90%. The entire still, exclusive of furnace and condenser, satisfied the logistics requirements for man packing, weighing 35 pounds in all-aluminum construction.

The bulk of the equipment was, however, disproportionate to the yield, and an effort was made to develop a more compact unit. Since the volume of water heated in the original design was greatly in excess of needs, consideration was given to a still of construction similar to a Liebig condenser. An elongated cylindrical evaporating surface with a concentric heating jacket proved the most compact for the largest heating area.

A copper test still was constructed with an evaporating cylinder 3 inches in diameter and 30 inches long surrounded by a closed water jacket 5 inches in diameter. To heat the jacket, wet steam from the building steam lines was introduced at 5 psi pressure at the bottom and exhausted at the top of the vertical jacket. A pipe trap outside the heated area was provided at the bottom of the inner cylinder to effect a vapor seal, and concentrates flowed continously from the trap overflow to an open container. Stream flow was adjusted so that all steam was condensed within the water jacket and the overflow condensate maintained at 100 C. The average yield of this equipment in recovered alcohol was 3 gph with maximum yields of 4 gph. The efficiency of recovery was 90% or greater (Fig. 11). The relatively greater portability of this type of still led to its adoption, in principle, for field operation.

Preliminary consideration was given to the construction of one or more stills of this character, heated by steam generated in a separate, portable boiler. Further studies led to the conclusion that a separate boiler would greatly increase the total weight of equipment, while affording no advantage in efficiency. Design was then standardized on a single still, incorporating an evaporating cylinder contained in a vertical fire tube boiler. The evaporating tube was $8\frac{1}{2}$ inches in diameter, heated over a length of 30 inches, which, based on yields of experimental units, was estimated to produce 8 - 12 gph of recovered alcohol. The boiler consists of a rectangular box 14 inches square and 36 inches long, with four $2\frac{1}{2}$-inch outside diameter fire tubes through its entire length, equally spaced around the central evaporator. The boiler is heated with a charcoal burning furnace, since this fuel is available in all cinchona producing areas. The evaporator tube is

provided with an easily removable cover for accessibility in cleaning, and with a distribution ring for spreading alcohol film over the surface. The condenser tube is a 15-foot length of 1¼-inch inside diameter, flexible, seamless copper steam hose, to be coiled in a fabric tank of cooling water. The still body, less furnace and condensing coil, satisfies logistic requirements, weighing 62 pounds and falling within dimension requirements (Fig. 12). All parts of the equipment except the condenser coil are of welded stainless steel. Complete construction drawings and details of the final model are included in Appendix D.

16. Field Drying Concentrates. Attempts to filter alkaloid concentrates as the end product of distillation shown them to be extremely difficult to pass through even the coarsest filter papers or cloths of normally tight weaves. The colloidal nature of the suspended matter in the liquid closes the pores of any filtering medium rapidly, and flow is greatly reduced or entirely prevented. On the basis of this condition, the following method for field drying of concentrates was formulated.

Medium weight muslin, or cloth of similar porosity, is stretched tightly over a series of rectangular wooden frames. The frames are assembled in a supporting rack, one above the other, so that one frame drains into the next, and a watertight container is placed to receive the drainage of the lowest frame (Fig. 12). As concentrate liquids from distillation are poured over the upper cloth in a uniform distribution, part of the liquid filters through to succeeding frames; thus a portion of the solids remains on each cloth, and the thin film of liquid retained evaporates rapidly.

When the cloths are covered with sufficient concentrates to reduce their efficiency or to become clogged, they are removed and the concentrates shaken and brushed from the surfaces. When colloidal material accumulates to the extent that it cannot be removed by mechanical means, the cloths may be taken from the frames and macerated in a dilute acid, or in alcohol. The resulting solution of alkaloids is reduced to concentrate by regular plant operation.

IV DISCUSSION

A. ACID EXTRACTION

17. Efficiency and Logistics of Acid Extraction. Acid extraction depends for its effect upon changing the alkaloid from its natural base, or such other form as it may assume while in the plant cell, into a salt of the acid used.

That the above reaction could be carried to completion, or nearly so, is obvious. That the efficiency of this extraction depends upon several factors such as the amount and volume of acid

used, and the length of time it has contact with the bark is also unquestionably true. In the presence of a sufficient excess of acid, the dibasic salt will be formed, in which state the solubilities, particularly in the case of the disulphates, are tremendously increased.

a. Time Factor in Acid Extraction. In considering the matter of contact time, two different aspects of the situation have to be explored. In one of these, dry bark is ground into subcellular sizes, in the other, the bark is cut or broken into "chips." In the former, aside from entrapment in the resinous mass which constitutes the cellular content of dried plant material, the alkaloids are freely presented to the solvent action of the extraction medium. Hence, the contact time need be no greater than that required to soften and disintegrate the gummy contents of the cell, plus whatever time the alkaloidal salt requires to go into solution. In extracting from "chips", however, the problem is entirely different. Here, in addition to dissolving the alkaloids out of the other cellular inclusions, the fluid must first place the cell walls into a condition in which they are sufficiently permeable to permit the passage of fluids both along the original vascular structure of the plant, and to some extent, transversally.

Having taken the soluble materials inside the bark cells into solution, the extraction process then proceeds to function like any other such system in which two solutions of different ion concentrations are set up on opposite sides of a permeable membrane. Osmotic action causes the fluid of lesser density to flow toward that of the greater; at the same time a migration of ions takes place in the direction of the lower ion concentration. Thus, in effect, there is flow in both directions. The osmotic flow will continue toward equilibrium at an ever diminishing velocity in one direction, while the ion migration takes place, also with a constantly diminishing velocity, in the opposite direction.

In the case of the ion migration, however, the end point is not clear-cut since, if the concentration of the "outside" phase be constantly reduced, an equilibrium cannot be reached. Instead, the migration velocity will steadily diminish toward a point at which it becomes no longer feasible to wait for completion of the reaction.

b. Particle Size. The above considers the osmotic system in terms of only one permeable membrane. However, in a piece of bark two inches long and one-quarter inch thick, a great many such membranes are interposed between the lumen of the cells in the interior and the point of lowest alkaloid ion concentration. In consequence the relationship between the adjacent cells as the fluid progresses toward the center of the "chip" is successively nearer to equilibrium and the ion migration rate at any one time is progressively slower

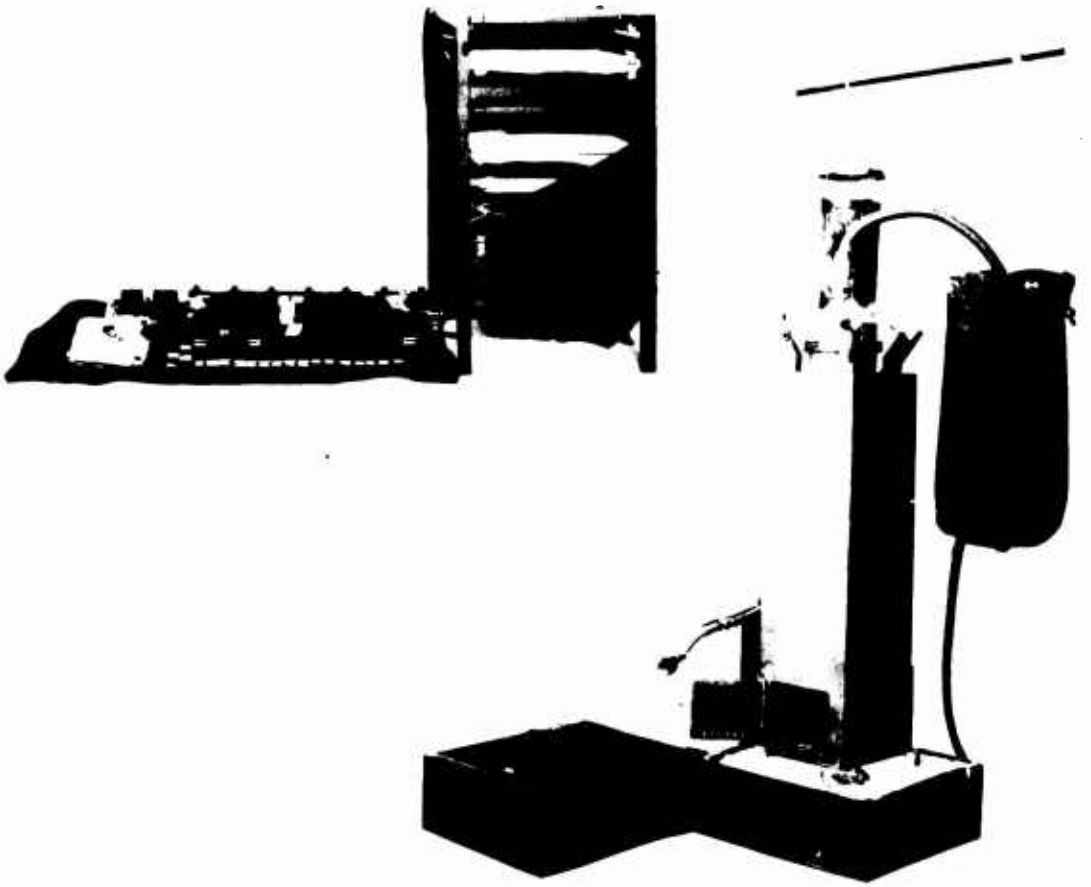

FIG. 12. ALCOHOL RECOVERY STILL AND CONCENTRATE DRYING FRAME

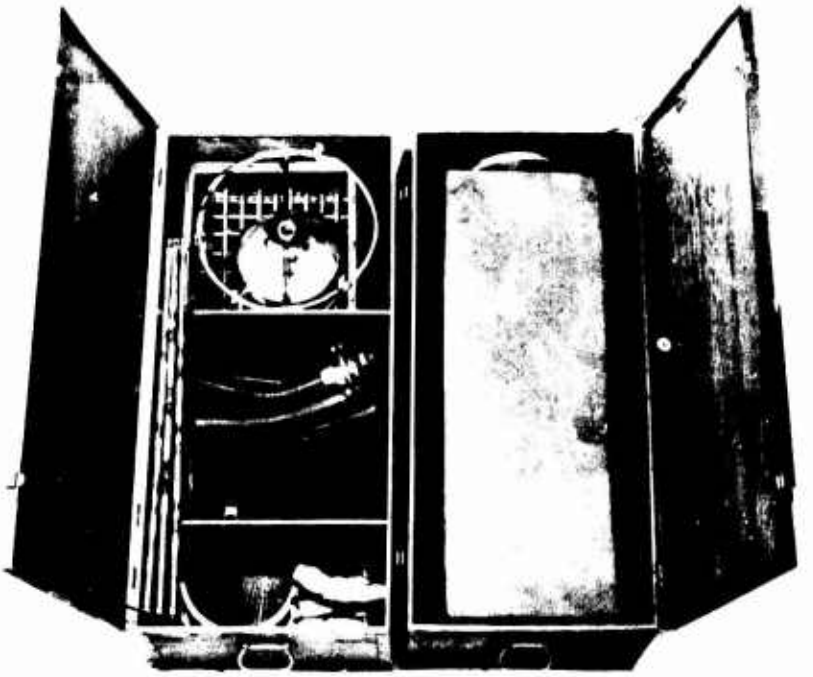

FIG. 13. STILL UNIT PACKED

as it is taken nearer to the center of the piece.

This diminishing rate might be diagrammed as in Fig. 14, the diagonal lines representing the ion migration rate and the shaded portions representing the unextracted alkaloid content in a given time(t).

From the diagram it can be seen that in progressively cutting the length of the bark piece in half, the amount of material remaining unextracted in a given time becomes increasingly smaller at a very rapid rate, since the distance from the center to outside is quartered with each cut so far as longitudiual distance is concerned. Thus, it is apparent that at some point, probably far short of the subcellular sizes of grinding, a condition will be reached wherein no appreciable value will be realized from further subdivision.

In the case of dry cinchona bark it may be argued with some logic that so long as grinding is to be done, it may as well be carried on to size 40 USBS mesh. Actually in passing dry bark through a 1/4-inch hammer mill screen it is found that as much as 85% will have been reduced to size 40 or less. The remaining 15% will be composed largely of wood splinters and individual cork cells, neither of which contains any considerable amount of alkaloid.

However, when considering a process involving the use of undried or "fresh" bark the problem of grinding assumes a much greater degree of importance. Though fresh bark can be ground to subcellular sizes by using one of the slurry grinding processes, it is found that, without exception, the machinery manufactured for the purpose is of a very heavy and cumbersome nature. It is questionable whether a lightweight machine of sufficient capacity for any quantity production program could be developed. In view of the foregoing it would be well to consider extraction from "fresh" chips as opposed to chips of dry bark.

In fresh plant material or even in material that has wilted but not dried, the tissues have retained a good deal of their original permeability. For this reason, a very brief contact with a supply of liquid will restore them to something very like their original condition. Further, the contents of the cell have not become hard and impermeable and, though some chemical change in these inclusions will probably have taken place in wilted material, it is likely that they will,for the most part, be in better condition so far as extraction is concerned. Macerations of "fresh" bark in the Latin America series of experiments (Appendix B) indicates that the above relation undoubtedly exists. What will be the optimum size of the fresh bark particles, can best be determined during the course of pilot plant operations in the field.

c. Chemical Requirements. In considering the logistics of the above reaction an arbitrary total alkaloid content of 5% of dry weight is assumed. Fresh bark having a moisture content of 66% will then contain, in 500 pounds of such material, 500 x .33 x .05, or 8.25 pounds of mixed alkaloids. At the average molecular weight of 310, this eight and one quarter pounds of alkaloid will represent $\frac{8.25 \times 453.6}{310}$ or 12.07 equivalents of alkaloid. To convert two equivalents of alkaloid to the sulphate, for example, requires two equivalents of sulphuric acid. If twice that amount of acid is present, the so-called bisulphate will form, thus increasing the solubility from 1 part in 725 to 1 part in 9. Thus, to reach its most soluble form, the alkaloid content of 500 pounds of fresh bark would require 24 equivalents of H_2SO_4 concentrate.

However, it has been found through repeated experiments that a large excess of acid is necessary to secure the desired extraction. It must, therefore, be assumed that the non-alkaloidal materials taken into solution are effective in using up a considerable portion of the available acid. The Latin America Experiments, in which several acid concentrations were employed, indicate that .1N acid, in sufficient volume to contain 48 equivalents, is the most effective concentration and volume for the maceration of 500 pounds of fresh bark.

18. Logistics of Alkali Precipitation Recovery Method. The cinchona alkaloids in the form of the natural base are difficult to dissolve in water. Quinine, the most soluble in this form, dissolves at the ratio of 1 gram to 1750 cc of water at 20 C. The alkali recovery method depends for its effect upon converting the acid salts in solution into the natural base form, with resulting precipitation of all but the portion which is necessary to fill the solubility ratio. The amount thus retained will, of course, depend on the total volume of fluid used.

a. Chemical Requirements. Through repeated trials, involving various acid concentrations, it was found that a sulphuric acid solution of about .1N concentration (1/2% by volume) produced the best extraction results (See Appendix B). To obtain adequate coverage of the bark plus sufficient excess fluid to permit agitation by means of a stream of the fluid circulated through the pump, 125 gallons of acid solution had to be used for each 500 pounds of fresh bark.

To precipitate the alkaloids from acid solution, enough hydroxide must be added to complete the following reaction:

$$H_2SO_4 + 2NaOH \longrightarrow Na2SO4 + 2H_2O$$

If 125 gallons of .1N solution are to be used per maceration the amount of sodium hydroxide required to complete the reaction can be computed as follows:

$$125 \times 3.78 \text{ (l/gal)} \times .1 = \text{number of equivalents} \times \frac{40}{453.6} =$$

pounds of NaOH. Substituting the proper quantities it is found that 4.17 pounds of the hydroxide are required to bring the above reaction to neutrality. At a pH of 7 (the neutral point) only a slight precipitation, consisting mainly of non-alkaloidal substances, occurs. To bring about complete conversion to the natural base, the full quantity as given above has to be introduced, plus sufficient excess to produce a pH of about 8.5 to 9. Near the end point of the reaction, the addition of small quantities of hydroxide may bring about a change greater than that required. Since the use of too much hydroxide causes the alkaloids to go into a colloidal state from which they cannot be either precipitated or recovered by filtration, it is essential that great care be exercised in not going beyond the point at which heaviest flocculation is observed. The proper pH value for maximum precipitation depends upon the nature and amount of material in solution, thus no definite rule concerning the required excess of hydroxide can be formulated.

b. Logistics. From the above it is known that 4.17 pounds of sodium hydroxide are required to neutralize 125 gallons of .1N acid. This amount of acid solution contains 5.1 pounds of sulphuric acid concentrate, or a total of 9.27 pounds of chemicals for one 500-pounds maceration. Six such macerations seem to be the minimum at which an acceptable extraction percentage can be reached. Thus, for continuous plant operation six macerations would have to be carried on simultaneously. In an operating month of 26 days, therefore, 1446 pounds of chemicals would be used in operating at a daily capacity of 500 pounds of fresh bark.

c. Efficiency. During the processing of 500-pounds of bark by this method 750 gallons of liquid will have been used. At the average solubility in water of 1 gram in 3105 cc, approximately 915 grams or about 2 pounds of alkaloid will remain unprecipitated and hence will be lost. On the basis of 5% total alkaloids in the dry bark this loss will amount to 25% of the available total.

The best extraction yet obtained, as gauged by an assay of the exhausted bark, is 76%. Assuming that in field operation an average of 75% efficiency in extraction could be maintained, the computed loss from material remaining unprecipitated would reduce this figure to 56% of the original total. A plant capable of handling 500 pounds of bark daily would then produce from 13,000 pounds of bark, 120 pounds of mixed alkaloids per month.

19. Logistics of Ion Exchange Recovery Method.

a. Maceration. As can be seen from the simplified reaction shown in paragraph 9b, above, the ion exchange recovery method provides for continuous renewal of the acid concentration of the menstruum during cycles through the ion exchange adsorbent. This effectively eliminates the necessity for changing the liquor during the maceration. With the exception of losses entailed in removing exhausted bark from the tank, no renewal of the acid liquor is required between different batches of bark. This loss, which on the basis of experience appears to be about 10%, places the acid requirement for one maceration unit for a 26-day working month at 325 gallons of .1N sulphuric acid. This amount of dilute solution requires 53.11 pounds of acid to operate one 26-day month if a three-day extraction period can be employed. A three-day maceration period will require three separate extraction units to operate continuously; for this reason, three times the above quantity, or 159.33 pounds, of concentrated acid will be required to operate continuously at 500 pounds per day capacity.

b. Regeneration. The quantities of regenerants, as experimentally determined by manufacturers of ion exchange materials are 5 lbs of 93% sulphuric acid and a chemically equivalent quantity of sodium hydroxide which is about 4 to 5 pounds per cubic foot of ion exchange bed, depending upon its chemical purity and moisture content.

Exchange capacities for several ion exchangers are variously reported at from 600 to 850 meq./l. Considering the lowest of these figures as the probable capacity, it then follows that one cubic foot of exchange material will have a calculated capacity of 16,992 meq. Experimental data taken on 200-ml ion exchange columns indicate that a substantial portion of the above capacity can be used. Ten grams of alkaloid were retained by a 200-ml ion exchange column. This, at the average molecular weight of 310, amounts to 32 meq. Thus, 5 x 32 x 28.32, or 4531.2 meq. of alkaloid can be held in one cubic foot of ion exchanger.

Returning to the assumed 500 pounds of fresh bark, at a total alkaloid content of 5% of dry weight it is calculated that there are 12,217 meq. of mixed alkaloids available. It follows that some 2.6 cubic feet of ion exchanger will be required to adsorb the alkaloids from 500 pounds of fresh bark. To allow a sufficient margin for counter current operation it is necessary to use three ion exchange columns of one-and-one-half cubic foot bed capacity, making a total for the unit of 4.5 cubic feet. However, since only 2.6 cubic feet of bed can be loaded with alkaloid each day, no more than two columns will have to be regenerated. Since 9 pounds of chemicals are required to perform both the acid and alkali regenerations on one cubic foot of bed, then 27 pounds will be required

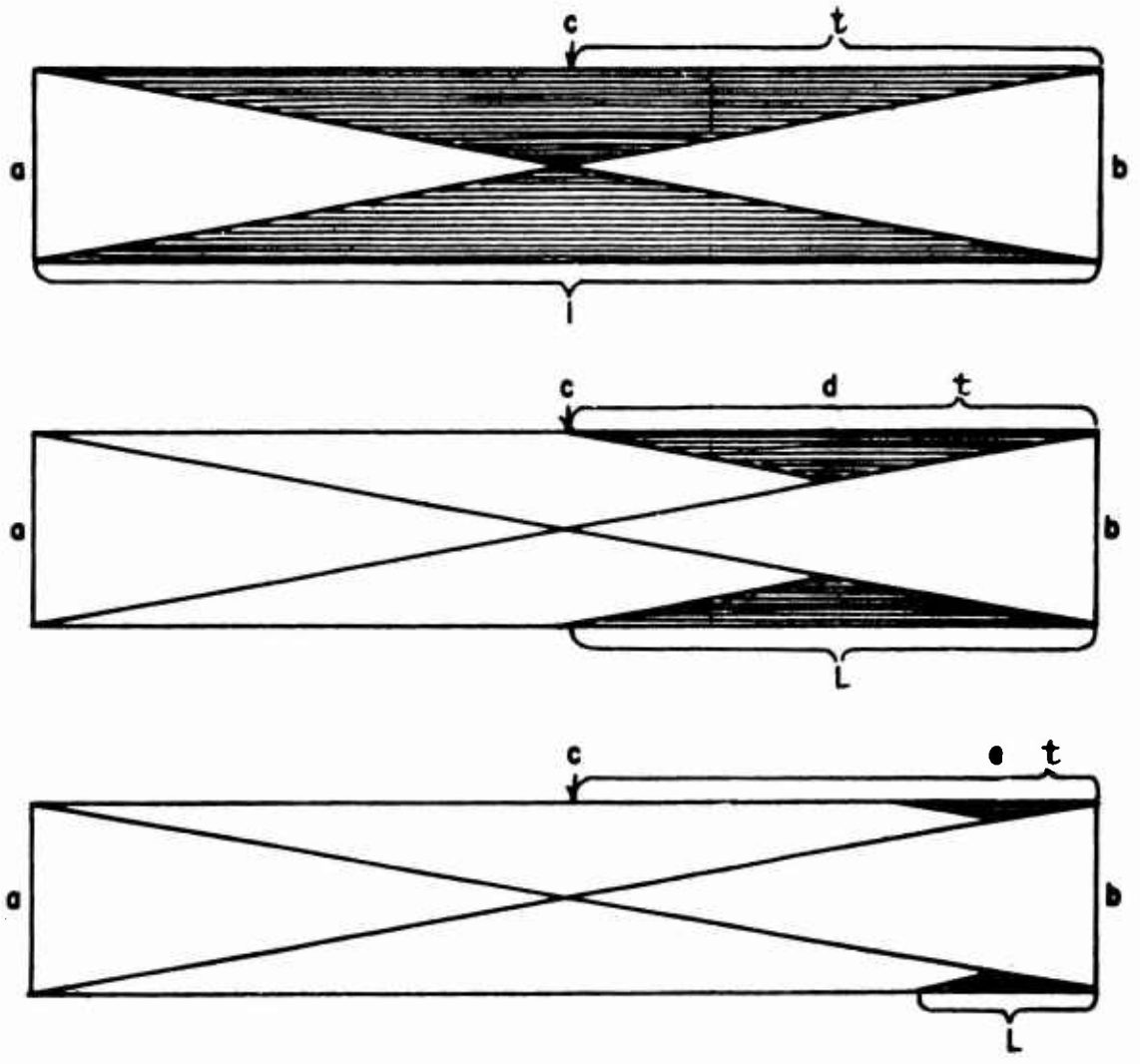

FIG. 14.

DIAGRAMATIC REPRESENTATION OF RELATION BETWEEN PERCENT OF ALKALOIDS EXTRACTED IN A GIVEN TIME (t) AND THE LENGTH OF THE BARK CHIPS (L). SHADED PORTION REPRESENTS UNEXTRACTED ALKALOID.

for daily operation and 702 pounds for a 26-day operating month. To flush two 1½ cubic-foot exchange beds with alcohol, as outlined in paragraph 10 c above, requires 10 cubic feet of alcohol. Losses in alcohol are, in general, about 5%, making the total loss for the operating month 13 cubic feet. From the above it is possible by computation to place the monthly alcohol requirement at 172.5 gallons, or 141 pounds, somewhat less than half of which will remain on hand at the end of the 26 days.

Totalling the above quantities indicates that about 1002 pounds of chemicals and alcohol are required to operate a 500 lb/day capacity plant for one 26-day month.

c. Efficiency. An examination of the recovery data for the EB2-8 series indicates that well over 90% of the alkaloids extracted can be realized in the end product. Thus, if an extraction efficiency of 90% can be maintained, and previous comparisons between fresh and dry bark extractions indicate that this may well be so, then an overall efficiency of above 80% will result. At this rate of efficiency, one 500 lb/day unit could, in 26 days of operation, process 13,000 pounds of bark and produce 171 pounds of mixed alkaloids.

B. PLANT SIZE

20. Large Scale Engine-Powered Plant. It is intended that the field extraction unit be set up as a continuous circulation system in which acid liquor is pumped from the maceration tank through a series of ion exchange columns arranged in tandem and employing upflow. From the last column in line the acid solution, by this time stripped of its alkaloid content, will be returned to the maceration tank. Though it is probable that downflow circulation will be employed within the maceration tank, the equipment lends itself quite as well to operation in the opposite direction.

From experience gained in the Latin American laboratory work, and more particularly from the recent scale model plant operation, the value of mechanical agitation in the maceration process is evident. The easiest means of accomplishing this function is obviously to utilize the excess capacity of the pump which circulates the liquid through the ion exchange system. This pump has a capacity of some 50 gpm, only about 4 gpm of which can be passed through the ion exchangers without damage to the beds. This leaves about 46 gpm excess capacity. It is found that a great deal of agitation can be promoted within the macerator by nozzling this stream down from 1 inch to 1/4 inch and directing it upward through the bark mass. This causes the entire contents of the tank to be displaced continuously upward at the center, with a resulting downward move-

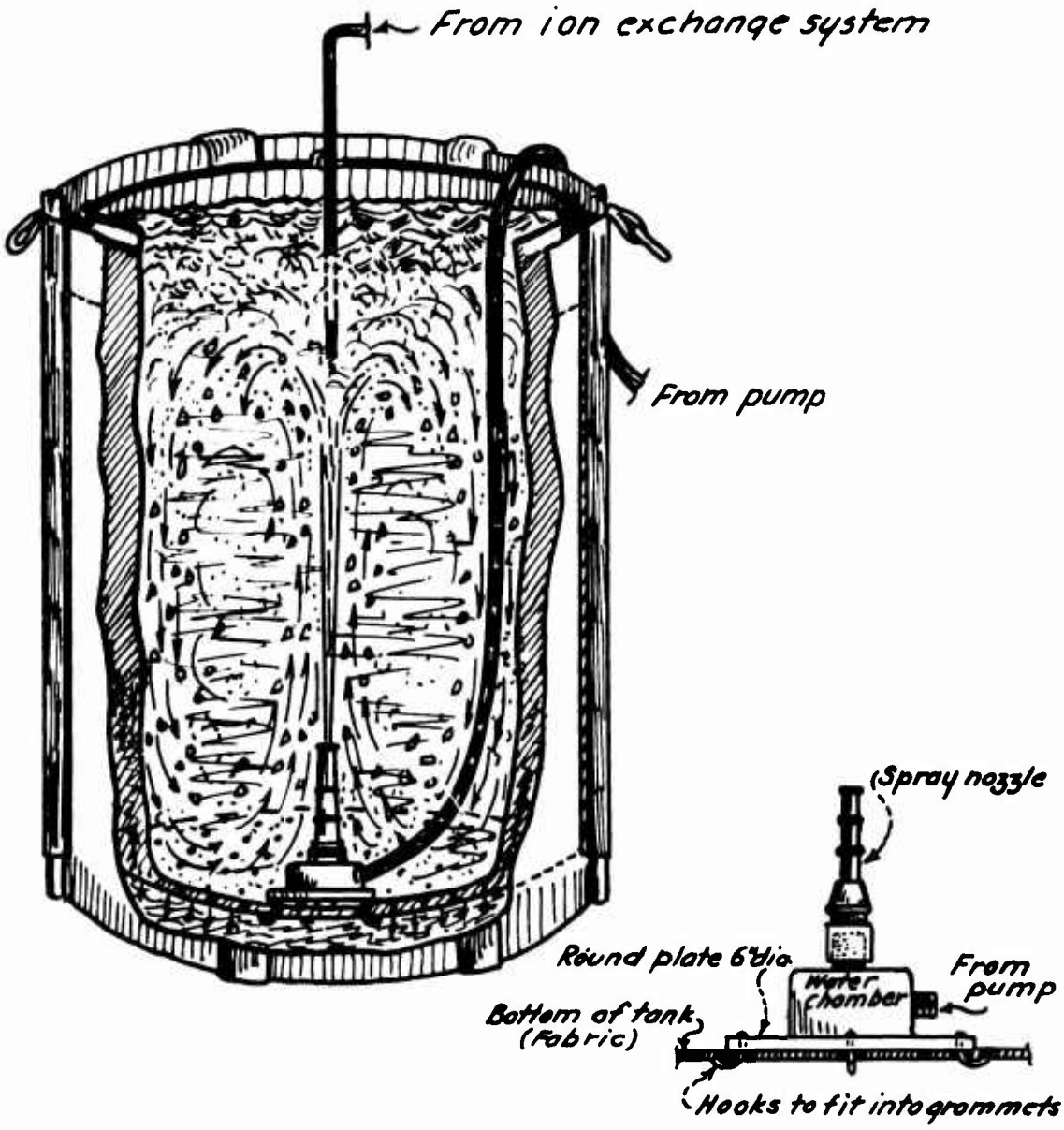

FIG. 15. AGITATION BY MEANS OF A DIRECTED STREAM

ment at the periphery (Fig. 15).

As shown by the flow diagrams (Fig. 2), the pump inlet is inserted between the wall of the tank and macerator. The major portion of the production of the pump is returned to the macerator as described above; the remainder, about $12\frac{1}{2}$ liters per minute, is circulated through the ion exchange system before being returned to the macerator, where it is directed to the center of the tank and dispersed by the rising jet from the bottom.

From the operation of the scale model it was ascertained that the rate of cation adsorption is greater than the possible rate at which alkaloids can be taken into solution from bark even under ideal conditions. It was found that the number of cycles of the acid menstruum necessary to reduce the alkaloid concentration of the acid to 1 part in 100,000, or less, varied directly with the amount of alkaloid remaining in the bark. While at the start, four to six complete cycles of the menstruum were required to accomplish this purpose, it was found that, by the end of the second such reduction in concentration, the maximum amount of alkaloid that could be taken into solution in the course of three to four hours was removable in two to three fluid cycles.

The above phenomenon fits in well with the plan of operating on three lots of bark simultaneously. That is, the first lot will be started and extracted for 24 hours before the second lot is placed in maceration, by this time the extraction rate of the first lot will have been so reduced that its menstruum need be circulated only occasionally. Similarly, by the time the third lot is started, the rate at which extraction can proceed in the second will have been greatly reduced, while the first lot will be approaching complete exhaustion.

Though some variation of the above will certainly prove to be the standard method of operation, much will depend upon the exigencies encountered in the field. Thus, the final operational plan will have to be elaborated through pilot plant operation.

21. Small Scale Hand-Operated Plant. For small scale production or operation in areas so inaccessible as to make the logistic requirements of gasoline engine operation prohibitive, a hand-pumped extraction plant can be assembled from the standard components of the basic Acid Extraction Unit.

This assembly constitutes merely a simple gravity arrangement of the cycling system described under paragraph 20, above. It employs an extra container at the outfall of the last ion exchange column in the system. This container, which may be one of the standard tanks or any convenient smaller vessel, serves as a reservoir for the hand pump which is to be used, at suitable intervals, for

the purpose of returning the percolate to the maceration unit. A suggested means of assembly is shown in Fig. 16.

Since this method of operation does not provide any means of agitation, it will be necessary to stir the mass of bark by hand from time to time to prevent channelling.

It is possible that this model may prove to be the most usable means of obtaining valuable and much needed supplies of quinidine, which is obtained from C. pitayensis, a species found only in the most inaccessible portion of southern Colombia and northern Ecuador.*

C. FUTURE WORK

22. Field Testing of Plants and Process. Since freshly harvested cinchona bark is unobtainable in the United States, all experimental work at the Engineer Board has been performed on dry bark. From the Latin America experiments it is apparent that considerably different behavior can be expected from fresh material; hence, it will be necessary to carry on the process where fresh bark is obtainable before a final report can be made.

Efficiency, durability, and portability of the pilot model extraction plant can be properly determined only under actual field conditions in the tropics. For this reason, it is planned that the equipment which has been designed and fabricated at the Engineer Board be placed in regular operation in a cinchona producing area by the officers of the Cinchona Research Unit.

23. Total Capacity Studies. Commercially manufactured ion exchange materials vary considerably in several ways. A series of total capacity studies designed to evaluate these differences has been under way for some time, but has not yet been completed. Since the above differences may assume considerable importance in their relation to the logistics of the entire process, these evaluation studies should be continued.

24. Field Manual. The adoption of the acid extraction method, particularly with regard to the use of small portable plants, would necessitate the training of a considerable number of operation and maintenance experts. To accomplish this purpose, a suitable field or training manual should be compiled during the period of pilot plant operation. Use of the manual in Latin America and the Philippines makes it essential that the publication be written in English, Spanish, and Portuguese. The manual should comprise a complete operations guide covering all essential phases of the chemistry, plant function, and maintenance.

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*Steere, William, "The Botanical Work of the Cinchona Mission in South America," Science, 16 February 1945.

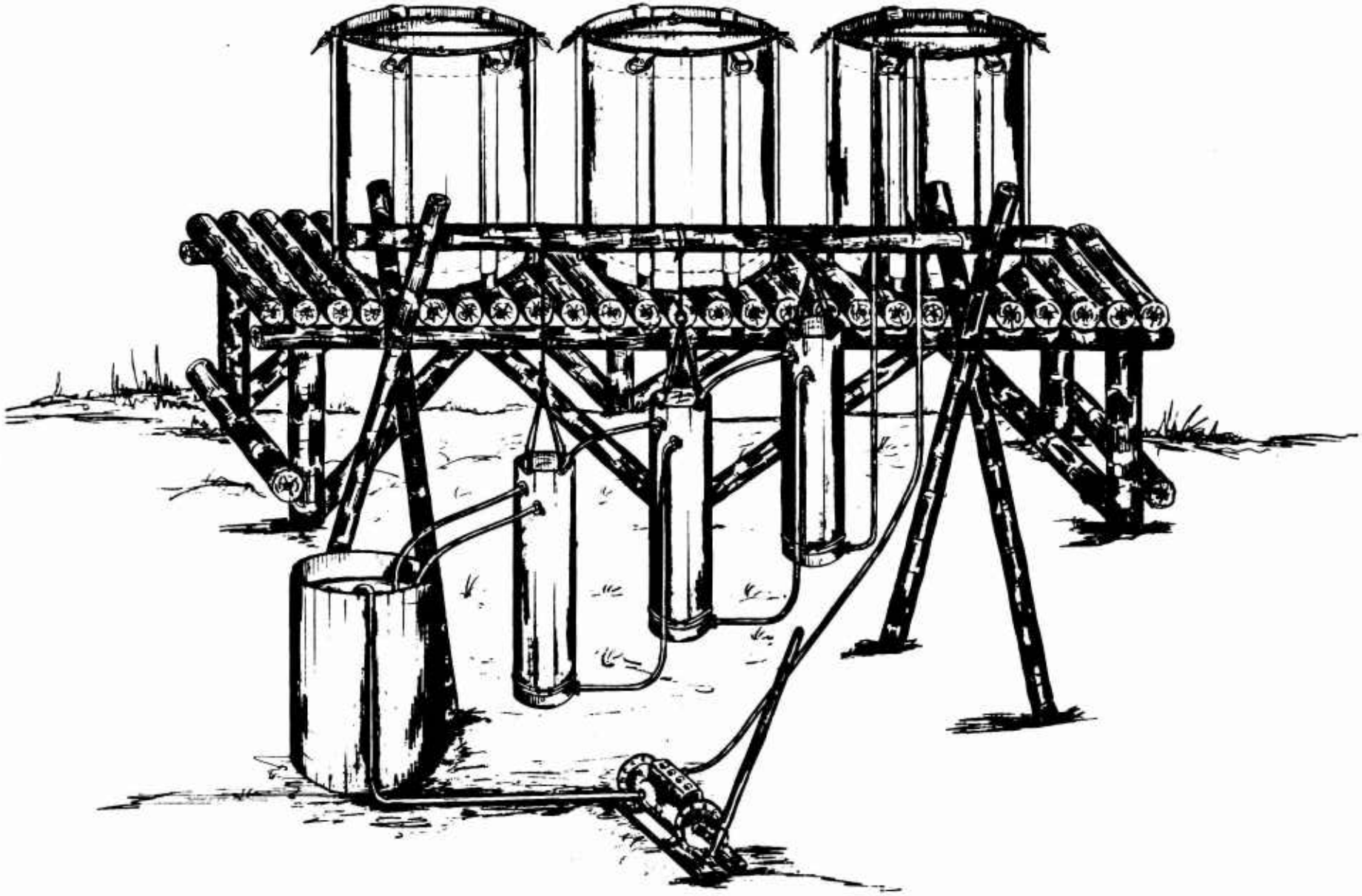

FIG. 16. FIELD EXTRACTION UNIT, HAND PUMPED GRAVITY FLOW MODEL

V. CONCLUSIONS

25. Conclusions. It is concluded, on the basis of the above and preceding experimental work, that:

a. Acid extraction of cinchona alkaloids, followed by ion exchange recovery, is an efficient process from both an economic and a logistic standpoint.

b. The process can be carried on in a completely portable extraction plant.

c. Acid extraction, followed by alkali precipitation, is a usable process where the efficiency and logistics of the method are not controlling factors, ie., a small unit operated for purely local consumption.

d. The end product of either of the above methods constitutes a usable antimalarial which may, depending upon the alkaloid content of the bark used, conform to U.S.P. standards for totaquine.

e. The acid extraction ion exchange process is directly applicable to the extraction of most, if not all, of the usable alkaloids.[9]

f. The component parts of the field unit have functioned satisfactorily when used individually for other comparable purposes; however, development of the extraction unit as a whole has progressed to the point where further modifications must be based on rigid field tests.

VI. RECOMMENDATIONS

26. Recommendations. It is recommended that:

a. The two existing pilot plants designed and constructed at the Engineer Board be subjected to a rigorous field testing program by the Cinchona Research Unit.

b. In conjunction with the above, a field manual covering the extraction process and the operation and maintenance of the portable plant be written during pilot plant operation and translated into the required languages.

c. Research on ion exchange capacities now in progress at the Engineer Board be carried to a conclusion.

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9. Appendix C, Consultant's Report.

Submitted by:

ROBERT LEE KAYE
Major, Corps of Engineers
Commanding Officer of Cinchona Research Unit

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APPENDIX A

RUTGERS REPORT

Illustrations

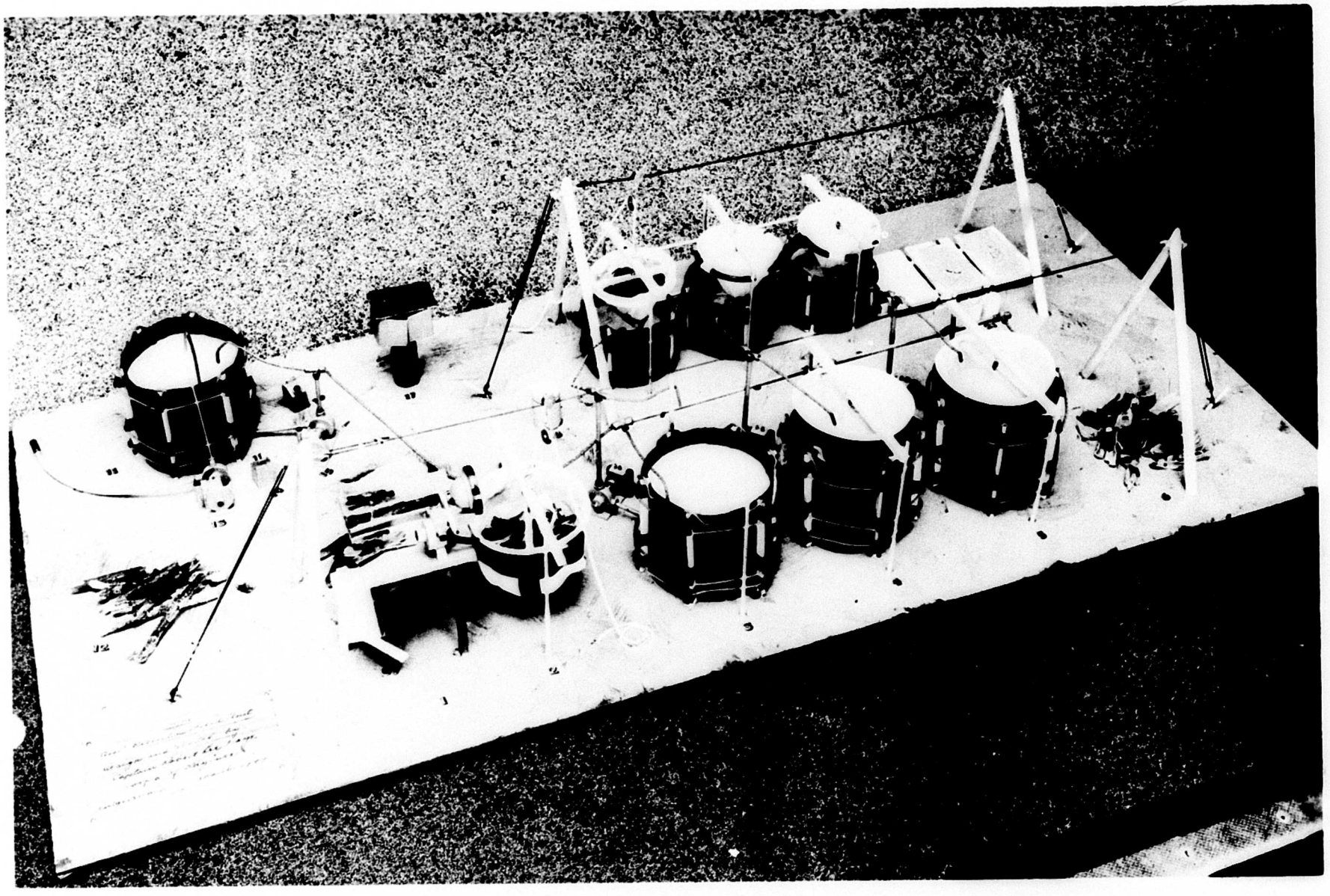


FIG. 17. EARLY CONCEPT OF EXTRACTION UNIT, DESIGN AND MODEL BY MAJOR ROBERT LEE KAYE

RUTGERS UNIVERSITY
Newark, N. J.

17 February 1944

Memorandum for Mr. Keith G. Cone, Chief, Cinchona Section, Miscellaneous Commodities Division, FEA:

Subject: Acid Extraction Pilot Plant

1. On December 10, 1943, final arrangements were made for laboratory space at Rutgers University, Newark, New Jersey. A program of research was established. This program included the information available from literature and the experiments conducted previously at Rutgers University. From this research, preliminary experiments were conducted to evaluate the various methods of acid extraction. Certain conclusions were arrived at and on January 1, 1944, a program of experiments was set up, certain laboratory equipment was purchased, and on January 15, chemists and assistants were hired. A series of fifty-five individual batch extractions were set up to ascertain:

A. An optimal period of contact of acid with dry bark

B. Number of washings of acid-treated bark to insure an economical yield

C. Amount and type of agitation needed

D. Methods of separating or de-watering the acid bark mixture

E. Method of recovering the alkaloids from the acid liquor

2. The above experiments were conducted under varying circumstances and using several acids with and without agitation. Each one of the fifty-five batches required from one to seven days and recordings were kept of each day's yield.

3. During these experiments, careful consideration was being given to the equipment necessary to construct a mobile unit that could be used at the site where the bark was gathered. A number of equipment manufacturers, including the manufacturers who are now supplying mobile field equipment for the Corps of Engineers, were contacted and several experiments were conducted in their laboratories.

3. Arrangements were made to have certain pieces of equipment loaned for trial at Rutgers University. At the present time, some of this equipment is still in use.

4. Until recently, all the experiments were conducted with ground bark of approximately 40-mesh. Again, with the thought of simplifying and minimizing the type of equipment necessary to do this job, it was decided that various mesh barks would be tried. Experiments were conducted with sizes ranging from 4-mesh up to chips as large as 2" x 2". The result of these experiments showed that the large chips produced a yield as great as the bark ground to 40-mesh with some decided advantages in the final precipitant. In addition to this, the handling of chips decreases the size and amount of equipment necessary for extraction.

5. All of the experiments were conducted using dry bark. At the present time, it is believed that fresh bark should give as great a yield, if not greater, than dry bark. Therefore, it becomes necessary to conduct a short series of experiments at the source of the bark in South America. In addition to this, several other determinations will be made, such as:

A. Optimal practical mesh size

B. Result of the use of fresh bark with this method of extraction

C. Effect of various preliminary treatments before and after grinding or shipping the bark

D. Type of materials available at the field locations

E. A complete study of time, yields, shipping, and handling

6. While this work is going on, the experiments at Rutgers will continue. With the knowledge obtained in South America and the work done at Rutgers, it will take a relatively short time to purchase and ship a complete plant to one of the locations in South America.

7. During the course of the experiments, several matters have turned up that may be of advantage to the general procurement program. One of these is a method of drying and packing the bark that is now being imported to the United States. Tests will be made along with the rest of the experiments in South America, and a complete report will be forwarded to the Administration.

Robert Lee Kaye
Captain C. E.

Rutgers University
1 Lincoln Avenue
Newark, New Jersey

ABSTRACTS FROM RUTGERS REPORT

The following experiments, (39 to 61) shown in tabular form comprise the major portion of the acid extraction work done at Rutgers University. Since experiments conducted prior to number 39 either do not bear direct relation to the problem at hand or have been insufficiently documented, they have not been included as part of this report. (Copies of original data sheets are available from the files of F. E. A.)

The maceration technique employed in this series consisted of moistening one pound of bark which had been passed through a 1/4 hammer mill (about 85% < 40 mesh) with the acid solution to be used in the maceration. Following the moistening process the bark was packed in a percolator, enough acid solution added to provide an excess and maceration allowed to continue for the prescribed period. At the end of the maceration, percolation was started. Three to four volumes of acid solution were passed through the bark in this manner, the volumes being saved separately. Each batch of percolate was then precipitated separately by raising the pH to 8 with NaOH. The precipitates were filtered off, dried and weighed with the filter paper which was used as an extraction thimble in the assay process so that it also entered the final gravimetric determination.

Experiments 39, 40, 60 and 61 were first moistened and macerated with acid introduced at boiling temperature and allowed to cool. The percolations were made with cold acid. The experiments using hot acid were performed on 2-pound lots of bark, all others on one-pound lots. Experiments 43, 44, 49, 52, 56 and 58 used $\frac{1}{2}$ percent H_2SO_4; 39, 42, 46, 48, 51, 55, 57 and 61 used $\frac{1}{2}$ percent HCl; and 40, 41, 45, 47, 50, 54, 59, and 60 used 1 percent HCl.

A simple but somewhat erratic method of determining percentage of extraction was used in experiments 39 to 61. The method consisted merely of extracting the oven dried precipitates on a soxhlet with chloroform. After extraction the residue in the extraction thimble was dried and the loss of weight determined. This loss was taken as representing the total alkaloid content of the precipitate. From this figure and an assay of the original bark, all extraction percentages were computed.

From experience gained in analysis of concentrates by the Dutch Method (see Appendix C, Dutch Method) it is obvious that results so obtained admit of a considerable margin of error. For this reason the experiments in qestion cannot be considered as having produced any quantitative or even comparable results.

However, they do indicate that a large percentage of the alkaloid content of cinchona bark can be extracted by means of simple percolation with dilute acids.

An experiment was conducted in which one pound of bark was macerated in a good grade of commercial vinegar for a period of three days. The maceration was followed by successive percolations with the same material. A yield of 16.2 grams out of a possible 25 was effected for an efficiency of 72 percent.

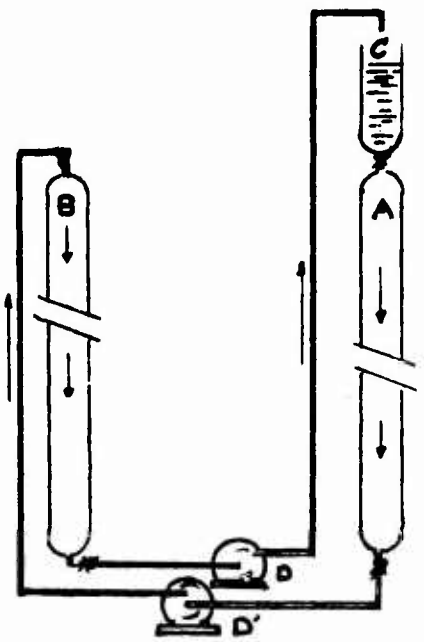
The extraction with commercial vinegar was conducted at the request of Col. Arthur F. Fisher, Director, American Cinchona Plantation. It has obvious value for small scale or emergency operations when commercial chemicals are not available. Caustic to complete this operation could readily be obtained by leaching ordinary wood ashes.

The report on ZeoKarb X Adsorption and a preliminary report by Mr. Norman Applezweig, Consultant, are the background against which the ion exchange recovery experiments at the Engineer Board were conceived.

LABORATORY DATA, RUTGERS EXPERIMENTS 39–61													
Experiment No.	% TA of Orig. Bark	Acid Used	Concentration of Acid %	Maceration Days	Ppt. Yields From Wash Nos.					Wt. of Alkaloid in Orig. Grams	Wt. of Alkaloid in Ppt. Grams	% of TA Recovered	Notes
					0	1	2	3	4				
39	1.24	HCl	1/2	1	5.39	18.78	–	–	6.63	19.8	1.9	9.6	Hot Acid, 2 Lbs. Bark
40	1.24	HCl	1	1	—	14.41		5.07	2.84	19.8	10.72	54	do
41	2.20	HCl	1	6	–	—	–	—	—	9.9	4.70	46	Cold Acid, 1 Lb. Bark
42	2.20	HCl	1/2	6	—	—	2.67	.56	.08	9.9	6.20	63	do
44	2.20	H_2SO_4	1/2	3	17.63	14.53	8.50	3.96	3.08	9.9	3.22	32	do
46	2.20	HCl	1/2	3	—	—	.68	1.02	.70	9.9	6.60	66	do
47	2.20	HCl	1	1	25.71	20.00	2.36	2.43	.67	9.9	5.43	54	do
48	2.20	HCl	1/2	1	8.40	5.10	4.20	3.06	2.31	9.9	6.60	67	do
49	2.20	H_2SO_4	1/2	1	—	15.88	10.38	2.82	2.78	9.9	6.95	70	do
50	5.60	HCL	1	6	7.41	—	—	—	.88	25.4	18.30	72	do
51	5.60	HCl	1/2	6	19.05	16.66	5.86	3.41	1.17	25.4	17.63	68	do
52	5.60	H_2SO_4	1/2	6	30.58	8.23	1.17	.86	61	25.4	12.99	51	do
54	5.60	HCL	1	3	32.17	11.07	—	1.03	.42	25.4	14.00	56	do
55	5.60	HCL	1/2	3	10.30	2.50	.90	.94	1.84	25.4	14.90	59	do
56	5.60	H_2SO_4	1/2	3	11.75	4.26	1.90	1.06	.98	25.4	8.28	32	do
57	5.60	HCL	1/2	1	24.70	13.06	3.60	3.03	2.52	25.4	16.53	65	do
58	5.60	H_2SO_4	1/2	1	13.20	13.42	—	3.02	1.94	25.4	12.93	52	do
59	5.60	HSL	1	1	18.63	16.54	5.54	2.28	2.37	25.4	19.57	77	do
60	5.60	HCL	1	1	21.44	10.98	19.47	8.59	4.20	50.80	20.62	40	Hot Acid, 2 Lbs. Bark
61	5.60	HCL	1/2	1	—	42.77	12.30	—	3.90	50.80	26.93	53	

FIG. 18. TABULAR RECAPITULATION OF RUTGERS EXPERIMENTS

ZEOKARB-X ADSORPTION EXPERIMENT



A - Cinchona Bark

B - ZeoKarb

C - Reservoir

D,D'- Pump

DIAGRAM OF APPARATUS

The ZeoKarb tube was prepared in the following manner to activate it:

The column was washed out with seven liters of 1 N. sulfuric acid by means of the circulation pump. An equal volume of water was then passed through the column. The speed of flow was about 500 cc per minute. The flow was then reversed, i.e., up through the column with an equal volume of distilled water, thus totally flushing the ZeoKarb-X and allowing it to resettle firmly.

Preparation of the drug for extraction:

Five pounds of the ground bark were mixed with ten pounds of wetted, washed sand, then wetted to allow the drug to swell. The moistened drug was packed into Column A and the apparatus was then set up and sealed. The menstruum, which was $\frac{1}{2}$ percent sulfuric acid, was added (10 liters) to the system until it filled it up completely excepting Column B (which was still wet with distilled water). Percolator C held excess menstruum. The drug macerated for twelve hours.

Extraction of the drug:

The water pumps were started and the top rate of flow was 120 cc per minute. This rate, however, gradually decreased

to an average of 80 cc per minute, thus taking an average of a little over two hours for a complete circuit of the menstruum through the system. The installation of the second pump was necessary to pull the menstruum into Column B from A, otherwise Column B would empty into percolator C too rapidly. The menstruum from Column B was tested periodically and was negative to Mayer's reagent. After 12 hours of running the menstruum from Column A was still positive to Mayers' reagent. At the end of 20 hours it was weakly so--at the end of 24 hours the drug was exhausted and gave a negative test with Mayer's reagent. The Column A was then unpacked and a sample saved for assay to check completeness of extraction.

Of the original 15 pounds of drug-sand mixture, only 8 pounds could be packed in the percolator, so the remaining 7 pounds were saved for another extraction and will be assayed as a check before extraction.

Comments

If the drug were mixed with more sand the rate of flow of menstruum could be increased so that the time for exhaustion could appreciably be lowered.

The pumps were erratic at times and refused to pump the menstruum, and at times would pump the menstruum from B to A faster than from A to B, therefore a constant watch was necessary to prevent the menstruum in Column B from falling below ZeoKarb-X.

PRELIMINARY REPORT

By Norman Applezweig - Consultant

It was necessary first to obtain basic facts as to the adsorption capacity of the ion-exchanger for cinchona alkaloids. Quinine was chosen as a representative alkaloid and capacity determinations were run on a 200 ml. ZeoKarb column under standard conditions, using quinine concentrations of 1:100 and 1:1000 and flow rates of 5 ml./min. and 50 ml./min. respectively.

In four experiments (1a, Ib, Ic, Id), it was found that the capacity of a 200 ml. bed of ZeoKarb for quinine from acid solution ($1S_oH_2SO_4$) was between 7 and 8 gms. before breakthrough. Total capacity figures were not obtained, but are believed to be at least 30 percent higher.

In experiments Ia through d, ammoniacal alcohol (10%) was used as a combined regenerant and elutriation solvent. In each case enough ammoniacal alcohol was passed through until a negative Mayer's test was obtained.

Experiments Ie and If, which are still in progress, are designed to determine the efficiency of a two-step process where an aqueous solution of ammonia or sodium hydroxide would be used as a regenerant followed by alcohol as a solvent.

COMMENTS

The data obtained thus far is of a preliminary nature but would seem to indicate the following:

1. Ion exchange equipment for cinchona alkaloid adsorption should be designed so that 2.5 liters of ZeoKarb bed are provided for every 100 gms. of alkaloid to be recovered.

2. Flow rates roughly equivalent to 25 ml./min. per liter of bed for concentrated solution (1:100) and 250 ml./min per liter of bed for dilute solution (1:1000) would seem to be satisfactory.

3. In a countercurrent system where the acid extract would pass through a series of exchangers, faster flow rates and higher capacities are to be expected.

4. Regeneration in experiments Ia through d showed that ammoniacal alcohol is a satisfactory "one-step" regenerant and elutriation solvent. However, a great excess over what was necessary was used. Actually, only enough ammoniacal alcohol should be used to regenerate the column and then sufficient alcohol should be added to dissolve the remaining alkaloids.

The two-step process using aqueous regenerant is being studied because it will facilitate the handling of the alcoholic solution of alkaloids. The presence of ammonia during the concentration of this extract being definitely objectionable. In addition, preliminary experiments have indicated that the aqueous regenerant removes an appreciable amount of color.

5. Sufficient data has now been obtained to enable the use of this process in recovering alkaloids from mother liquor.

A series of acid extraction experiments should now be run in which all mother liquors are run through ZeoKarb columns and the alkaloids recovered and added and compared with those obtained by alkaline precipitation.

6. The results obtained thus far seem to incidate that the ion exchange technique should prove a very valuable supplement to the acid extraction of cinchona insofar as the overall efficiency may be improved by the recovery of alkaloids which would otherwise be lost in the mother liquor following alkaline precipitation of the acid extracts of the bark.

A second series of experiments was performed for the purpose of evaluating the use of ion exchange technique in purifying the crude totaquine obtained by alkaline precipitation of acid extracts of the bark.

A standard totaquine, prepared by alkaline precipitation of an acid extract of the bark, assaying 23.4% total alkaloids and of a brick-red color, was used in these experiments. Twenty-gram portions were heated and stirred with 500 ml. of .7 N. H_2SO_4, filtered and used as follows:

In experiment IIa, the extract was passed through a fresh 200 ml. of ZeoKarb at 5 ml./min. The effluent was found to be of the same color as the original, positive to Mayer's reagent, but showed no precipitation on the addition of alkali. (The original extract exhibited substantial precipitation upon addition of alkali.)

The column was regenerated with 20 ml. of NH_4OH (23%) in 200 ml. of alcohol and was followed by alcohol until negative to Mayer's test (total 500 ml.). The alcohol was removed by evaporation and the residue made alkaline with NH_4OH and shaken with $CHCl_3$. The major portion of the color remained in the aqueous phase and a light yellow colored chloroform extract was obtained. This was evaporated under an infra-red lamp, leaving behind an almost white crystalline residue of approximately 2 grams. This was assayed and found to be 94% total alkaloids (Method is very crude and believed to be ±5%).

In experiment IIb the same process was repeated except that the effluent from IIa was used to dissolve the totaquine and the column was regenerated with 363 ml. of 1 N. NH_4OH. A mixture of 3 parts alcohol and 1 part chloroform was used as an elutriation solvent, about 1250 ml. being required. The solvent was evaporated under vacuum, the residue made alkaline and shaken with $CHCl_3$. Upon evaporation of the solvent under an infra-red lamp the residue was allowed to remain for too long a period and was scorched. The weight was disregarded, since upon assay a considerable amount of carbonized material was evident. However, the preparation assayed 84% T. A.

In experiment IIc a twenty-gram sample was again dissolved with the aid of heat and stirring in 500 ml. of .7 N. H_2SO_4 (undissolved residue approximately 9 gms.). The column was regenerated and treated as in IIb and a white crystalline residue was obtained weighing approximately 2.4 gms., which assayed 91% T. A. Note: No moisture determination was run.

COMMENTS

1. According to these preliminary results it would seem that ion exchange represents an excellent technique for purifying crude totaquine preparations, improving solubility (and consequently adsorption in the digestive tract), appearance, and removing non-alkaloidal, non-ionic-contaminants to give a pure alkaloidal preparation.

2. Further experiments are necessary before the efficiency of the method can be calculated, however a recovery of at least 52% has been demonstrated. Since the preparation studied proved so insoluble upon attempted redissolution in acid, it would seem that a major portion of the alkaloids might have been lost by oxidation or complex-formation in the original totaquine precipitate.

Further experiments should be made using freshly precipitated totaquine and more careful study should be made

of the methods for preparing the totaquine solution prior to passage through the column. The totaquine solution should also be assayed prior to use.

3. Additional knowledge regarding the two-step regeneration-elutriation technique has been obtained in this series, which would seem to indicate that this method is as efficient as the one-step, ammoniacal alcohol method. The results confirm the fact that only a minimum quantity of ammonia is required for actual regeneration.

A mixture of chloroform and alcohol seems to show no advantage over the use of alcohol alone.

4. Information available at this time is that conventional purification techniques (redissolution in acid and precipitation by alkali or shuttling between acid and solvent) rarely results in more than 25% alkaloid recovery.

APPENDIX B

LATIN AMERICA REPORT

Illustrations

July 8, 1944

TO: David Adler, Chief
General Commodities Division

THROUGH: Colonel Arthur F. Fischer
War Department Liaison Office

FROM: Robert Lee Kaye, Capt., C. E.

SUBJECT: Report of Acid Extraction Experiments

As a result of intensive studies of conditions, research, experiments, surveys, and field tests, we feel we have developed a new acid extraction process and a practical low-cost mobile field unit. These units are to be located close to the supply of cinchona bark. The extraction of concentrated alkaloids are leached from the fresh stripped bark without the necessity of drying and grinding. Each unit is capable of processing three hundred to five hundred pounds of bark per day. According to the South American survey, this is the average amount gathered in any one location per day. The unit is designed to be mobile and can be transported by mule pack. With little experience a unit can be installed and ready for operation within forty-eight hours after a location has been cleared and water is available. The equipment and the process were designed to operate with a minimum amount of labor. Each unit will require six average laborers and one supervisory foreman. A unit should be located where there is a minimum of thirty days supply of bark. When this is exhausted the unit is moved to the next location and operations are resumed.

The chemicals required for the process are common and readily available. Each ton of bark requires less than one dollar's worth of chemicals to produce the alkaloidal concentrate. Each ton of bark produces approximately sixty pounds of concentrated alkaloids. (This figure is dependent upon the original content of the bark.) Therefore, instead of transporting a ton of bark only sixty pounds are shipped.

At the present time this method extracts 75-80% of the alkaloids present in the bark. However, there are indications that this can be increased. Using 75% as a figure and comparing it with the figure of 90% as reported by American manufacturers, the net results are about equal. Figures to substantiate this are found in the experimental reports from South America which are attached.

Although a great many acid extraction methods have been developed, this is the first time within our knowledge that a mobile unit has been designed to extract alkaloids from fresh stripped bark without drying and grinding.

It is estimated that these units can be purchased in quantities of ten or better for approximately $2500 per unit complete in the open market. Considering the elimination of the cost of drying, grinding, packing, freight, and the various losses which occur in the present method of handling, the cost of a unit can be amortized in a relatively short time.

Using this new method makes it possible to select the kind and grade of bark to be processed, and if applied, will eliminate the many bottlenecks now encountered in South America. As an illustration the following quotation comes from the Ecuador survey report.

> "The chief limiting factor in area #1 is the extremely wet climate which makes it impossible to dry bark on the spot. Approximately one-half of the bark produced in this area is now transported a distance of ten to twenty miles to a desert area where drying is possible. If the drying were eliminated production could be doubled."

At the present time the purchase price of bark is determined by assays made with dry bark. This method is always subject to dispute because of the variance that can occur in making these assays. By the use of this new method bark would not be purchased; instead, the concentrated alkaloid would be contracted for. The assaying of the concentrate is much simpler and more accurate, and therefore, would eliminate a great deal of the dispute between producer and purchaser.

In making the survey through Latin America, the American Embassies and the offices and laboratories of the Foreign Economic Administration were visited. The plan was explained and discussed; and as a result of these conferences it is suggested, for political as well as practical purposes, that units be put in operation simultaneously in Guatemala, Colombia, Ecuador, Peru, and Bolivia. By operating these units simultaneously, it will be possible to observe conditions in the various countries and the results obtained, which information will be readily available for use in determining future policies and programs.

All of these units should be of the mobile type with the exception of the one in Guatemala. There the unit should be of a more permanent nature with a processing capacity of one ton of bark per day. In view of this recommendation and the present situation in Guatemala, it is suggested that the harvesting of bark be reduced to one ton per day.

The cost of the first units are estimated at $5,000 per unit or a total of $25,000. In Guatemala it is planned to use the existing equipment to make up the difference of cost between a mobile and a permanent one-ton unit. These units would be useful in demonstrating this advanced method of extraction to the many private producers.

This process will contribute toward the continuation of cooperative relations between the United States and Latin America. It places in the hands of the many people now engaged in the stripping and handling of bark

a new method that can be continued, and which provides an inexpensive means of producing anti-malarials which can be distributed or sold very cheaply to the native population. In addition to this, the United States will be in an advantageous position for obtaining a large supply of quinine rapidly whenever necessary.

This process will be of immeasurable value to the permanent plantations now being fostered by the United States in Latin America. By the time the bark from these plantations is ready for harvesting, the process should be greatly advanced, and the acid extraction method should become an accepted part of the procedure necessary for producing quinine from cinchona bark.

There are several methods that can be employed to purify the alkaloid concentrate. It can be shipped to the United States or used by Latin American manufacturers who are now engaged in processing dry bark. By the elimination of large bulk materials these manufacturers can increase the production of their own end product several times over at a reduced cost. It is suggested that these various manufacturers be shown the units in operation and the advantages pointed out to them. They may be interested in setting up and controlling a number of these mobile units themselves.

A great many of our experiments were conducted at Rutgers University using dry bark. With the select information obtained from these experiments, we repeated them in South America using fresh bark. The experiments conducted in South America proved successful. Attached to this report are copies of the experiments conducted at Rutgers as well as those conducted in the field. Also attached is a survey report.

We cannot help but feel that by our method lower costs and consequently a wider distribution of anti-malarials through Government and private means, would be effected, and that this would materially aid in improving the health conditions of laborers in Latin America; thus adding many man-hours to the production of other products necessary to supply the industries of the United States with vital materials.

REVIEW OF ACID EXTRACTION RESEARCH PROGRAM

These notes and comments are based on an exhaustive examination of all laboratory reports, the Latin America Survey, conversations with Colonel Arthur F. Fischer, members of the Cinchona Mission of FEA, several cinchona producers of South America and others connected with or interested in the Acid Extraction Research Program. Recommendations for further research and reasons for such are included.

The Problem:

Until the Japanese occupation of Java and the Malay peninsula all save an insignificant quantity of the world's supply of cinchona alkaloids was produced in that area. These alkaloids are available in South America and at various times in the past large amounts of cinchona bark have been exported from that source. However, by 1941 Latin American production of cinchona bark had fallen off to a very low level.

Several small alkaloid extraction plants have been operating in Latin America for many years, some of them having been set up as a part of the League of Nations totaquina program. However, the maximum production capacity of these plants is not sufficient to supply more than a small part of the antimalarials needed for local consumption. With the cutting off of the Asiatic Zone as a source of cinchona alkaloids, it became necessary to exploit the South American stands of wild bark to their maximum capacity in order to obtain the enormous supplies of antimalarials required for military operations in malarious areas. The resultant slaughter harvest of these stands and its attendant difficulties gave rise to several problems.

1.) The ever-increasing difficulty of transportation from point of harvest to shipping point. This problem grows progressively more acute as the more accessible stands are depleted and operations carried farther from available road facilities. In several regions work has progressed so far into the mountains that bark is being transported for two days or more on the backs of men before it reaches an access road. In one region at least there are two men engaged in transporting bark for every one that is engaged in actual harvesting. This, in addition to trebling the cost of harvest, creates a labor problem which limits production to about one-third of the potential capacity of the region.

2.) The rapid exhaustion of readily available supplies of high grade bark suggests another problem. Though the existing supplies of usable bark in South America have by no means been fully

exploited it is fairly obvious that if the Western Hemisphere is to have any assurance of being able to supply its own needs in this field, some effort will have to be made to keep Latin America in the cinchona business. This can take the form of the development of plantations or of providing some means whereby wild stands can be exploited on an economical sustained yield basis. Since a considerable time lag is to be expected in the development of producing plantations both means may well have to be employed, at least temporarily.

3.) According to a report issued by the National Research Council, the greater part of the bark so far harvested in South America is low or lacking in quinidine and that for this reason, existing stocks of this essential drug have fallen to dangerously low levels. In an attempt to alleviate this shortage, the National Research Council, among other things, has requested the Foreign Economic Administration to exploit the quinidine-bearing cinchona species of South America to the utmost.

The most promising species for this purpose is probably Cinchona pitayensis. The region in which pitayensis is found extends from the Nevada de Huila in Colombia to about the latitude of Quito in Ecuador. Exploration is as yet incomplete but it appears that the species occurs in scattered stands on the three cordilleras of Colombia and the two of Ecuador throughout this entire belt, at altitudes above seven thousand feet.

The unfortunate fact that the above region is one of the most inaccessible yet explored, plus the habit of the species of growing in extremely scattered stands, almost as isolated plants, creates an unusually difficult transportation problem which must be solved before any great quantities of quinidine can be obtained from this source.

4.) Not the least of the considerations involved in the Latin America cinchona industry are its diplomatic aspects. The most immediate, though not necessarily the most important of these has to do with the maintenance of good will on the part of the cinchona producers. Agencies of the government of the United States have put many Latin Americans into the cinchona industry and encouraged them to expand. Most producers, giving heed to previous experience in this field, have persuaded themselves that when the present war emergency has passed the cinchona supply will be permitted again to fall into the hands of those who previously controlled it, and that Latin American producers will, as before, be unable to compete. This may be one of the reasons why Latin American capital has been so sluggish in this field. If the cinchona alkaloid market is permitted to seek its own level then the only way to keep the Latin American producer in business will be either to subsidize him or to enable him to put the industry on a basis that will permit him to compete. One way of accomplishing the latter would be to provide an extraction method which would be cheap, simple efficient and tend to minimize the problems of transportation and labor.

Another way in which Pan American relations might be served would be in the providing of a method whereby the Latin American countries could produce a cheap antimalarial for their own use and in quantities sufficient to meet their own requirements. Here again an inexpensive plant that is mobile or portable seems to be indicated.

5.) In addition to its function in solving the production and social improvement problems of South America, a portable alkaloid extraction plant could play an important part in the rehabilitation of cinchona producing areas in the present war zone as they are recovered from the enemy. This was recently pointed out by Colonel Fischer in a conversation concerning the reconstruction problem faced in the Philippines.

INITIAL WORK IN ACID EXTRACTION

In 1941 Major Kaye and a consultant chemist undertook to develop a feasible method of acid extraction. Their experiments, using the standard grinds of ordinary commercial dried bark, were intended to explore the possibilities of developing a cheap method of extraction for low grade cinchona barks. Conversations between Major Kaye and Colonel Fischer concerning the exorbitant cost of transportation and the need for an inexpensive febrifuge for local consumption led to the consideration of the acid extraction method for high alkaloidal barks as well.

At this same time an independent series of experiments was being carried on by Mr. Martin Ulan of the faculty of Rutgers College of Pharmacy. Mr. Ulan's work, also on acid extraction was directed toward the development of a quick method of quantitative alkaloidal determination that would be adaptable to use in the field as a guide to purchasers of cinchona bark. (Rutgers report). In December of 1942, Mr. Ulan having heard of this parallel program arranged, through the office of F.E.A. Cinchona Section, for a conference with Major Kaye. Upon comparing notes, it was decided that it would be advantageous to pool their efforts and at the request of the Foreign Economic Administration, Major Kaye was placed on temporary duty at Rutgers for this purpose. Shortly thereafter it was decided to explore the possibilities of a hypothesis postulated by Major Kaye that the inherent inefficiency of the acid extraction method would, if the process were operated as a field unit and used fresh (undried) bark, be more than offset by the losses that commonly occur during the drying and shipping of the commercial product. It was further postulated that by extracting to a highly concentrated substance at the source, a real saving

in the cost and effort of transportation would be realized. To determine a feasible field technique and explore the matter of plant design a program of preliminary investigation was set up at Rutgers College of Pharmacy under the co-direction of Major Kaye and Mr. Ulan.

EXTRACTION TECHNIQUE

In the course of investigating various methods of maceration the question of the proper size of grind was raised. It was reasoned that in acid extraction, as in any osmotic system, ionic exchange will take place between the two phases until an equilibrium is reached. Though it is obvious that by this method one could never attain complete exhaustion of the alkaloids it appears none the less probable that a satisfactory percentage could be thus extracted without the necessity of changing the solvent solution, i.e., lowering the ion concentration of the outside phase too many times. The speed of this reaction will be governed by the size of the pieces of bark and the permeability of the cell walls. In this latter respect "fresh" bark should be more easily extracted since its cell walls still retain their highly permeable nature. It has been demonstrated that as high as 76% of TCA can be extracted from one-half inch chips of fresh bark of an original TCA of 4.3%.

In all macerations performed at Rutgers ordinary commercial cinchona bark was used. An attempt to secure a reasonable approximation of fresh bark by shipping preserved material from the finca at El Porvenir in Guatamala proved unsuccessful, so it was necessary to conduct a further series of tests in the cinchona producing countries of Central and South America using freshly harvested bark.

Several series of experiments were run at Rutgers, some of them duplicated with fresh bark in Latin America for the purpose of determining:

1. Kind of acid best suited to the process.
2. Optimum concentration of acid.
3. Required length of maceration period.
4. Required number of changes of solvent solution.
5. Effect of stirring, circulation of solvent, etc.
6. Influence of heating on speed and efficiency of process.
7. Efficiency of an ion exchange process.

1.) The tests as to kind of acid best suited to the purpose involved the use of sulfuric, hydrochloric, acetic and a solution of commercial vinegar. Though satisfactory extractions

were made with all of these, sulfuric was finally chosen as having the best all around qualifications. That it can be transported in its concentrated state by means of steel containers was considered of prime importance because of the necessity of packing into the jungle.

The success with commercial vinegar suggests the possibility of using locally available materials in areas where commercial chemicals are difficult to obtain.

2.) Experimental work in the matter of acid concentration covers a range from 3 percent H_2SO_4 by volume to 0.5 percent HCl by volume, the first producing 5.4 times the hydrogen ion concentration of the latter. While much work remains to be done in order accurately to determine the most effective acid concentration, experience so far indicates that 0.5 percent H_2SO_4 by volume gives as good a performance as any of the stronger concentrations used.

3.) Macerations have been performed in which the period varied from 12 to 96 hours. However, the introduction of several variables such as changing acid concentration, heating, mechanical agitation etc. have prevented the accumulation of data which would provide any truly conclusive answer to the question of proper time interval.

It has appeared that macerations in which the solvent was circulated and fresh solution applied at 24-hour intervals obtained the best results.

A series of experiments set up at Rutgers for the specific purpose of determining this function failed in its purpose due to faulty analytical technique and must be repeated.

4.) Experience in the matter of the required number of washes seems to indicate that four or more solution changes should be made regardless of the length of maceration time or other factors involved. However, it seems that this should certainly be a function of maceration time and acid concentration so further study is indicated.

5.) Stirring, circulation, or a combination of the two have, without exception, increased the speed and efficiency of the process. Though the performance of such a function presents difficulties in connection with operation of a field unit, its necessity seems inescapable and satisfactory methods will have to be worked out.

6.) The application of heat would be very desirable from the standpoint of the relative solubilities of the alkaloidal sulphates, however, the use of any effective heating mechanism presents a most difficult problem in connection with field operation. Thus the desirability of heat application should be fully established before the engineering problem is considered.

7.) In April of this year some investigational work in the matter of an ion exchange method of alkaloid extraction was undertaken at Rutgers. Mr. Norman Applezweig acted as consultant. The first experiments set up in this program were for the purpose of determining a suitable extraction-recovery technique. A workable system was outlined on the basis of several experiments in which quinine sulphate was dissolved in acid solution and passed through a 200 ml. column containing ZeoKarb, a product of Permutit Company. These experiments were followed by Exp. 37 (Rutgers Report) in which a 4-inch by 48-inch ZeoKarb column was used. It was planned here to determine percentage of recovery on the basis of actual alkaloids produced by distillation of the solvent rather than by the usual assay of the residual bark. That both determinations should have been employed became apparent when, through neglect on the part of a laboratory assistant, the alkaloids were destroyed by overheating in the final stage of recovery.

A second large scale ion-exchange experiment was set up in which a laboratory water purification apparatus loaned by Permutit Company was used. In this experiment involving the continuous circulation of a small quantity of acid through a ZeoKarb-cinchona bark system, complete extraction of the alkaloids was secured in 24 hours of circulation. The completeness of the extraction was taken at the point at which the acid solution proved negative to Mayer's reagent. This reagent is sensitive to about one part in 100,000. Since at twenty hours the acid was only weakly positive to Meyers, it may be conservatively assumed that extraction at this point was better than 95 percent.

This experiment was left in operation by Lt. Ronzone when he was ordered to join Major Kaye in Guatemala. It is regrettable that Mr. Ulan found it necessary to discontinue the experiment before its completion.

A preliminary report on the ion exchange method which was submitted by Mr. Applezweig, the laboratory report on the interrupted Rutgers experiment* and a logistic analysis of these two reports compiled by Lt. Ronzone are attached to this review.

From the above it appears that further research is warranted on nearly every phase of the acid extraction method. That these should be undertaken according to their relative importance is obvious. That sufficient time and facilities to undertake all the necessary investigation in the immediate future will probably not be available also seems evident. Accordingly proposed investigational work might well be divided into three categories; information essential to successful field operation,

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* See Appendix A

work that will point the way toward logical improvement of the field plant, and data useful in consideration of the possible modification of the method relative to the nature and purpose of its operation. The following is a proposed plan of experimental procedure and priorities for each category.

1. This experiment falls outside the seven factors influencing operation listed above insofar as its prime purpose is concerned. It is intended as a dress rehearsal of the acid extraction-alkali recovery method as presently conceived and consists merely of a full scale extraction using 166 pounds of dry commercial bark (the equivalent of 500 pounds of fresh bark). During this experiment problems connected with circulation, filtering, setting up of apparatus can be ironed out. That a partial answer to the question of the number of solvent changes required to affect maximum extraction can be obtained is incidental. This extraction should be run to completion and actual recovery correlated with extraction as gauged by assay of the exhausted bark.

2. An ion exchange extraction should be undertaken on the same scale as the first experiment. It is believed that one such extraction would provide the answers to all major problems concerning the ZeoKarb method.

3. The third experiment should consist of a series, run in sufficient replications to produce reliable data on the subject of the acid concentration-maceration period relation. It should be run on a laboratory scale and subject to the most rigid controls.

The above are the experiments considered necessary to place the project in a position to start field operations.

The second group should include a series applying circulation, a second based on the principle of stirring, a third involving the application of heat and trials of various combinations of these factors.

It is now planned tc conduct the above work at the Engineer Board Water Purification Laboratories at Fort Belvoir, Virginia. Steps have been taken toward obtaining the required approval from the Office of the Chief of Engineers.

It should be pointed out that this process is by no means limited to the extraction of cinchona alkaloids. As a matter of fact it should lend itself readily to the extraction of other alkaloids such as scopolamine as well as many other plant extracts of a non-alkaloidal nature. Many of these products are of considerable strategic importance in view of the character of certain military undertakings.

It is believed that the acid extraction process will prove to be of considerable value in the commercial field, to the C.I.A.A. in the establishment of their health program in Latin America and, according to discussions with officials of the Department of State, would be of measurable aid in the furtherance of both present and future cordiality in inter-American relations.

LOGISTIC ANALYSIS OF ION EXCHANGE EXPERIMENTS

It was originally assumed that the use of ZeoKarb adsorption as a means of recovering cinchona alkaloids from the solvent in acid extraction of fresh bark was precluded by (a) the weight of the ZeoKarb bed itself, (b) the amount of ammonia gas (NH_3) and alcohol that would have to be carried, (c) the necessity for distilling the alcohol in the final step of recovery.

In view of the fact that complete extraction of dry cinchona has been obtained in a 24-hour circulation it would seem worth while to re-examine these suppositions and compare the results with the known logistics of the alkali precipitation-filter method of recovery.

In examining the first of the above suppositions it is assumed that the plant will operate on 500 pounds of fresh bark per day. This amount of fresh bark is roughly equal to 166 pounds of dry bark. Using 5 percent bark, one pound of ZeoKarb will retain the alkaloids from two pounds of dry bark. Thus, to extract the 500 pounds of fresh bark of the above alkaloidal content, 83 pounds of ZeoKarb would be required.

Since extraction can be completed in 24 hours it would not be necessary to carry more than two of the 500-gallon tanks. If the process can be accelerated by increasing the volume of circulation (laboratory circulation was at the rate of only 50 to 80 cc per minute) or a draining table provided upon which the exhausted bark could be placed in order to recover as much of the solvent as possible, one tank only might suffice. However, assuming that two tanks are used and that an alkali precipitation unit could operate to full capacity with eight tanks, this would do away with 500-gallon tanks or 600 pounds of plant equipment. This provides 600 pounds toward an alcohol still, the ZeoKarb bed and additional equipment such as a motor generator unit to operate the still. It is not possible accurately to determine the weight of such equipment but, as can be seen from the above, the weight of the ZeoKarb bed would be no great part of it.

Since in the ion exchange method the acid is continually swept clean it does not lose its solvent power, so the only expenditure of acid would be that amount that could not be drained

from the exhausted bark when it was removed from the acid bath. Placing this loss at ten percent, which is probably high, the loss in acid will amount to .062 gallons of H_2SO_4 conc. per day or in a 26-day operation period, 12.09 pounds. This amount plus the 1.2 pounds required to make up the original solution gives a total of 13.29 pounds H_2SO_4 conc. required for one month of operation. No NaOH is used in the process so a complete saving is realized in this category.

It is possible, from an examination of Mr. Applezweig's preliminary report, to arrive at a more or less accurate estimate as to the volume of ZeoKarb and alcohol that would have to be used in the operation of a 500-pound capacity field plant.

From the above report, "Ion exchange equipment for cinchona alkaloid adsorption should be designed so that 2.5 liters of ZeoKarb bed are provided for every 100 gms. of alkaloid to be recovered". Thus, at 5 percent TA a 94.11 liter bed would be required to remove the alkaloids from 500 pounds of fresh bark, this would amount to about 24 gallons, which indicates that a relatively small container could be used. To flush and strip a 200 ml. ZeoKarb bed 500 ml. of ammoniacal and untreated alcohol were required. Expanding these figures to a 94.11 liter bed indicates that some 23.75 liters or about six gallons would be required to strip a bed that would retain the alkaloids from 500 pounds of fresh bark. This figure is probably low but even at twice that amount distillation would be no great problem. There is no basis in the report for computing the amount of ammonia gas that would be required in the regeneration of the column.

An idea as to how the ion exchange process might be compressed to require smaller quantities of recoverable materials is suggested by the small filter units on hand at the Engineer Board. These units will hold about five gallons and due to their high speed filtering system would make ideal ZeoKarb beds. The possibility of using three such small units suggests itself as a means of reducing both the amount of ZeoKarb and alcohol required. A small ZeoKarb bed could be used until loaded and then cut out of the system, another being started in its place. While the second bed is being loaded, the first is being stripped, reactivated and the alcohol distilled. Other small beds can be held in reserve. It may thus be possible, through continuous operation, to use about one-half the quantities of ZeoKarb and alcohol indicated by the above figures.

The ion exchange method of recovery would, in addition to vastly cutting down the required amounts of chemicals, also cut the weight of the end product to the actual alkaloids only. One must consider the fact that using 5 percent TA bark, in one month of operation at 500-gallon capacity and 80 percent efficiency,

about 600 pounds of concentrate would be produced. According to Dr. Walde's totaquina assays, two-thirds of this amount or 400 pounds would be materials other than the sulphates of cinchona alkaloids. The ion exchange method would produce from this same bark 250 pounds of pure alkaloids which would meet USP standards.

The ion-exchange method, if it can be worked out on a basis satisfactory for field use will provide the necessary increase in efficiency of the extraction-recovery system, provide for the recovery of chemicals, will probably cut down the weight of the actual plant.

Further efforts to cut down on the weight of the plant suggest reducing the size of the tank from 500 to 250 gallons, hooking two small centrifugal pumps to one motor unit, changing plan for derrick rig to some simpler mount for the chain block which will require more manual effort.

To simplify the process it might be possible to accomplish all circulation and changing of liquids by means of hand pumps, thereby eliminating the gasoline motor which, to say the least, is not very well understood by the South American peon. All quantities of acid and other chemicals will have to be set up on a simple volume basis.

Silvio E. Ronzone,
1st. Lt. C.E.

(From a memorandum to Capt. Kaye)
Washington, D. C.
6 September 1944.

THE SULFURIC ACID EXTRACTION OF REMIJIA BARK

Object: To determine the effect of the sulfuric acid extraction of alkaloids from green Remijia bark and to recover the alkaloids from the resulting solutions.

Procedure: Five Lister bags were hung from the ceiling of a small room, the two bags (Nos. 1 and 2) containing the bark to be extracted and the one surrounding the bag containing the precipitate (no. 4) were stationary. The other bags (Nos. 3, 5 and 6 were adjustable and could be raised and lowered by the use of a block and tackle.

Hereafter the bags will also be referred to as numbers.

Arranged in the following manner as illustrated:

(5)	(1)	(6)
(4)	(2)	(3)

Nos. 1 and 2 contained 50 libras (500/454 lbs. U.S.= 1 libra) each of bark. No. 3 was used for making up the acid solution, and circulating the liquors in Nos. 1 and 2. No. 5 was used for precipitation of the alkaloids. No. 6 was used the first day for circulating the liquor. It was transferred to position No. 5 for convenience in transferring the precipitate to No. 4.

Preparation of bark for the bags: It was intended that the bark should be cut with a machete and screened through a 1/4-inch mesh screen. However, because there was no wooden floor available or a suitable screen, and it was necessary to get the green bark in contact with the acid as soon as possible, three people were set to work breaking up the bark into small pieces about one-inch square. The bark was then placed on a concrete floor and stirred until it was decided that the material was uniformly mixed and a representative sample could be taken. Then at least twenty-five small portions from all of the pile were taken from the pile in order to assure a uniform sample. About three pounds of bark were taken from a sample for analysis. This was placed in a wide-mouth jar of about one-gallon capacity, and then sealed tight. It was kept twenty-four hours at room temperature (about 70°F). It was then realized that there might be a change in alkaloid content if the green sample had to be kept until the end of the experiment so it was placed in the electric refrigerator to keep it cool until the end of the experiment. (Five days later)

Fifty libras of the original mixed bark were placed in each of two cotton bags which were suspended from bags No. 1 and 2.

The acid solution was prepared by putting tap water in bag No. 3 and adding 1/2 percent by volume of concentrated sulfuric acid (approximately 96% pure). The bag containing the acid solution was raised with a block and tackle and the solution allowed to run by gravity until each bag contained enough solution to cover the bark. About twenty-five gallons of solution

were required for each bag. This will vary somewhat with the amount of space around the inner bag containing the bark and also the height of the bag above the bottom.

For each acid change every twenty-four hours, eighteen gallons of water were necessary per bag.

Sampling the acid-extracted bark: At the end of each twenty-four hour extraction, the liquid was removed and a sample taken of the bark. In order to get as nearly representative a sample as possible, about twenty-five small portions of bark were taken from the top half of the bag. Samples were taken from the top, towards the center, and also from various places near the outside. About a one-pound sample was removed. This was placed in a small cloth bag and the sample washed free of excess acid until it was neutral to wide-range indicator paper.

The samples were spread out on a piece of cloth on the floor to dry overnight and the next morning they were placed in a paper bag, properly identified.

The acid solution was circulated once each morning and in the afternoon it was transferred to bag No. 5 by gravity. The bag was then raised off the floor, the solution neutralized with concentrated caustic (to a pH of approximately 7 (less than 8) wide range indicator) and then allowed to settle overnight. The next morning the supernatant fluid was decanted and the precipitate was transferred to the cloth bag which was used as a filter. The first part of the filtrate comes through cloudy and the solution is saved to be returned to the filter. As soon as the filtrate is clear the liquor is discarded.

The filter bag containing the precipitate was disconnected and taken outside to dry. Only the first two precipitates were kept apart. The third, fourth and fifth were combined. The precipitates were placed inside at night.

Preparation of caustic: Stick caustic soda was placed in beakers and enough water added to make about 25% solution. Stir well to complete solution. It should be prepared some time before neutralization of the acid, because a cold solution is less dangerous and more easily handled. A concentrated caustic is used in order to keep down the volume of solution.

Precipitation: Previous to this experiment, an arbitrary pH of 8 had been selected on the theoretical basis that the free bases would have to be completely precipitated at this acidity. This had been tried with a mixture of the sulfates of quinine, cinchonidine and cinchonine. However, it was observed at once in this precipitation that the precipitate was actually going into a colloidal solution and that the precipitants would not settle properly. A few preliminary tests were made which showed that the cloudy filtrates containing some of the precipitates would form a clear solution on the addition of a small amount of alkali. The solutions were tested for complete precipitation. A precipitate which settles properly shortens the time of filtration very markedly. In the first place, more liquid is removed by decantation and second, the solution filters better and there is a considerable saving in the time of filtration.

Filtration: The filter bags were prepared by seqing large cotton bags twice down the center lengthwise and then cutting them into two long narrow bags. The bag was folded back over the outside so that two thicknesses of cloth acted as the filter except that at the bottom only one thickness was necessary. When the bags were taken out to dry, the outside layer of cloth was folded away from the inner layer in order to hasten the drying of the precipitate.

These precipitates are very fine and have colloidal properties in water retention about like that of ordinary clay. So large that they will hold water several times their own weight.

The time in removing the water (decantation and filtration) is such that the precipitation bag (No. 5) can be emptied each morning and be ready for the next change of acid solution in the afternoon.

Size of room: 8 ft. x 12 ft. x 10 ft. high. Since inside supports had to be built inside the room and block and tackles were used for raising and lowering the bags, it is necessary to operate in a room that has at least a 10-foot ceiling. If the room is lower, the bags cannot be raised sufficiently to transfer the solutions from one bag to another by gravity.

There were no drains in the room, so final filtrates were removed from the bottom of the filtration bag (No. 4) by a small rubber hose. The whole of the experiment for five days may be carried out without wetting the floor from any of the solutions.

Drying the precipitate: Because of the colloidal nature of the precipitate and the high water retention, the drying of the precipitate is very difficult. Previous experience has shown that these precipitates cannot be dried at a high temperature (around 65°C.), because as long as there is considerable water present the material forms a sticky syrupy resin-like precipitate which hardens on cooling.

A small portion of the precipitate was dried at about 40°C and it seemed as though this temperature is sufficiently low to prevent resin formation and hasten the drying of the precipitate. Once the precipitate is air-dry, the product can be heated to a much higher temperature, because the physical properties of it have changed.

Water Supply: Sufficient and cheap water should be readily available so that the acid solutions may be prepared without any person carrying water. The use of a rubber hose for transferring water to the bags is suggested.

Method of analysis: The benzol method of extraction of the bark and the precipitate was used. For total alkaloids the Dutch method was used, which consists of adding 10 milliliters of standard one-normal hydrochloric acid to the boiling flask, then 25 to 30 cc of distilled water, removing the solvent, filtering and back-titrating at almost a boiling temperature with cold standard one-normal sodium hydroxide. An average molecular weight of 310 was used in the calculation. A more accurate and better method of analysis of total alkaloids is the cold ether-chloroform gravimetric method, but it would have lengthened the time considerably in obtaining the results. When only total alkaloids were wanted, a four-hour soxhlet extraction was made on the bark samples.

The benzol method of extraction is intended for use on cinchona barks. There was no modification of the method adapted for analysis of precipitates such as this. The ordinary USP totaquina method of analysis cannot be used because the material is not acid-soluble under the conditions of the method. For this reason it was necessary to use special techniques in the preparation of the dried precipitates.

After, the precipitates were air-dried and weighed. Since it was desired to keep the precipitates as such for future reference, they were not ground. One percent of each of the precipitates from each bag was taken in order to get a representative for analysis. If it becomes necessary at a later date to analyze the individual precipitates, this may still be done.

Five gram samples ground to pass a No. 40 mesh screen were used. Six grams of powdered calcium hydroxide and 3 cc of water were added. Cotton was used to separate the sample into individual portions to insure complete and more rapid extraction and also to prevent the sample from forming a hard mass in the thimbles, which would result in incomplete extraction and low yields.

Duplicate analyses were made on the original sample and the final samples. Single analyses on those in between were made because this would interfere less with the regular operation of the laboratory.

In the preparation of the original bark for extraction, four determinations were made: two with sodium hydroxide and two with water instead. No significant difference in results was obtained.

Miscellaneous notes: It has been calculated that there was present each day about six times as much sulfuric acis as was necessary to dissolve all the alkaloids in the original bark.

The iron chloride test for cupreine on the tartrates obtained from the analysis of the precipitate was negative.

The bark was dried at about 45°C, then ground to pass a 40-mesh screen. After five days' extraction, the liquors were still highly fluorescent, which phenomena indicates the presence of the quinine series of alkaloids.

EXPERIMENTAL DATA

PERCENT OF TOTAL ALKALOIDS IN REMIJIA BARK
(Uncorrected for moisture of acid solubility)

Days Extraction	Sample No. R - 1 % T.A.	R - 2 % T.A.
1	1.69	1.54
2	1.09	1.22
3	1.07	1.22
4	.99	.91
5A	.95	.79
5B	.90	.79

0 (Original composit samples)	2.45 2.53

PERCENT OF EXTRACTION
(Not corrected for moisture or acid solubility)

Days Extracted	R - 1	R - 2
1	34	38
2	56	51
3	57	51
4	60	63
5A*	62	68
5B*	64	68

* A and B are duplicates

MOISTURE CONTENT OF ORIGINAL GREEN BARK DRIED AT APPROXIMATELY 45°C is 67.1%.

ANALYSIS OF PRECIPITATE
(By the benzol method)

FIVE GRAM SAMPLES A AND B ARE DUPLICATES

	A	B
Quinine anhydrous alkaloid	9.97%	10.49%
Quinine sulfate heptahydrate	13.41	14.12
Cinchonidine alkaloid	2.48	1.79
Cinchonine	4.16	4.12
Quinidine	Traces	Traces
Sum of four alkaloids, so-called "TCA"	16.61%	16.40%
TOTAL ALKALOIDS (Dutch titration method)	27.13%	26.56%

Average 26.85%

WEIGHT OF PRECIPITATE (AIR DRIED)

Number of Precipitate	Grams
After first day	507
After second day	265
After third, fourth and fifth days	469
Total	1,241 grams (2.48 libras)

Percent yield of precipitate on the dry bark basis 7.5%

Discussion of results: By assuming that the alkaloids are not preferentially extracted, an assumption which is based on previous analyses of C officinalis and C pubescens barks, the amount of alkaloids recovered from the bark represents 2.0% alkaloids on the dry bark basis. (The original was not determined for quinine because at the time only total alkaloids were being considered for analysis. It can be calculated that by combining the tartrates from the two samples there would be scarcely enough for the customary .5 gram sample.)

The fact that Remijia barks contain a large portion of the alkaloids as quinine marks the precipitation high in quinine content, as high as is required for totaquina USP XII. Even though it does not contain as much of the other alkaloids.

Suggestions for future work:

1. Make an acid extraction of green Pitayensis bark, particularly of one containing a high quinidine content. Such a product may produce a highly desirable product for recovery of quinidine, a very expensive drug for use in heart ailments such as auricular fibrilations.

2. Accurate small-scale experiments should be set up to determine the amount of total solids which go into solution. It is possible that much more non-alkaloidal material is being dissolved than is assumed. When this is done the percent of extraction can then be calculated more accurately and this will raise the apparent percentage of alkaloid extraction based on bark analysis alone.

3. To economize on acid and save in the amount of work, it is suggested that after the first two days the acid be left in contact with the bark for a much longer time.

4. Make other extractions of Remijia barks that are now considered as too low to be used for recovery of quinine. It may be an important source of quinine as well as cupreine, in an emergency. Cupreine is a very expensive and imported drug and is made presumably by the demethylation of quinine.

SUMMARY: A remijia bark which was calculated indirectly to contain approximately one percent of anhydrous quinine alkaloid (2.5% TA) has been acid-extracted to yield a product that represents only 7.5% of the original dry bark and has a quinine sulfate heptahydrate equivalent of about 13.8%.

PRELIMINARY VINEGAR AND ACETIC ACID EXTRACTION EXPERIMENTS

Object: To see if vinegar or acetic acid has possibilities in replacing sulfuric acid in the extraction of cinchona alkaloids.

Procedure: Only one gallon of poor grade vinegar could be obtained. Its appearance was much like that of a suspension of clay, in water with a large amount of settlings on the bottom. Since ordinary vinegar is about 3% acetic acid it was decided to dilute it two times to make about a 1% solution. A sample was saved for analysis, which analyzed 2.84% acetic acid by titration with standard alkali. This made the resulting solution about .95% in acetic acid. The volume of solution that could be used each day was calculated and then sufficient Remijia bark added that it was still nicely covered. The solution was poured off each day for five days and new acid added each time. The bark at the end of five days was washed free of acid, and dried. Temperature of the acid extractions about 70°F, size of pieces about one inch square.

Experimental data:

Extracted Bark	A.	1.43% T. A. (Dutch Titration Method)
	B.	1.47% T.A.
Original Bark	A.	2.45% T.A.
	B.	2.53% T.A.
Average		2.49% T.A.
Amount Extracted		43.% and 41.%
Average		42.%

Discussion: Acetic acid was used in order to see if the extraction could be made with a cheap source of acid independent of a foreign supply. The vinegar cost one peso (57 cents U.S.) per gallon. In Bogota good table vinegar could not be bought in bulk but only in bottles of about a pint for one peso each. The bark turned black or purplish during the extraction, as though the vinegar were contaminated with iron salts. The bark acted as though it were actually going through a process of fermentation. The pH of the solution was about 3.

The solution that was decanted was neutralized with caustic soda in the regular manner and the precipitate was purple as though it was contaminated with iron salts. The precipitate could not be made to flocculate as nicely as by the sulfuric acid method. For example, the solution from the second decantation was neutralized and 550 cc placed in a 500 cc graduate. After standing overnight the precipitate was settled to 100 cc. The precipitate was purple and not very flocculant. The solution above was not at all clear and considerable colloidal matter remained suspended in the solution. Another experiment was set up with 2.% of glacial acetic on officinalis bark. In this case the solution was not changed until the end of four days. (Temp. 17-19° C Approx.)

The bark had been put through a hammer mill and the material was smaller than that which would pass through a 1/4-inch mesh. The original bark contained 6.13% total alkaloids whereas the acid-extracted material had 3.60% and 3.64% of total alkaloids in duplicate analyses, or an average of 41% of the total alkaloids was extracted.

The solution in this experiment was much different from that of the previous experiment. The material on precipitation with alkali gave a nice light-colored precipitate which flocculated and precipitated nicely. The acid-extracted bark did not have the black color as was found in the previous experiment.

Summary: Since the experiments were only of a preliminary nature, the results are not conclusive. The low yield of extraction would indicate that vinegar is not a suitable extraction medium for removal of these alkaloids. The high price of vinegar is also a disadvantage of its use for this purpose.

MEMORANDUM

June 22, 1944

To: Capt. Robert Lee Kaye

From: Arthur W. Walde

Subject: Acid Extraction of Cinchona Bark

Method of Procedure

Sulphuric acid was used in all experiments ½ and 1% by volume. The green bark was chopped to pass a 3/4 mesh screen and placed in blue "granite ware" pails. Enough acid solution was added to cover sufficiently the chopped bark. Representative sample of the original green bark was dried and analyzed for both TA and TCA. The exhausted acid treated samples were analyzed in a similar manner. At the end of each cycle the excess acid or water was removed by pouring off as much of the liquid as possible and then re-charging with an equal amount of similar solution. In experiments 11 and 12 alternate acid-water treatments were used. Out of 2500 cc of solution used in each pail, 2100 cc were removed and a like amount replaced to bring the solution to the original volume.

The exhausted barks at the completion of each experiment were thoroughly washed three times with water to remove excess acid. The wet sample of bark was then spread out thin and allowed to dry over the boiler of the heating plant at the Institute. The temperature on top of this boiler varied between 45° and 50° centigrade. From 2/4 days were necessary for the samples to be sufficiently dry to be milled to pass through a size 40 mesh sieve.

The solutions were filtered to remove small particles of bark, dirt, etc., and 400 cc of each were neutralized to a pH of 8.0 to 8.4 and the volume of precipitates was compared. The acid attacked the lining of the pails and enough iron was dissolved to produce a dark blue or "inky" color when the solution was neutralized. Presumably the color is due to an iron complex with the soluble material from the bark. Iron tannates are known to produce similar colored solutions.

In the last two lots of bark identified by the P (pubescens) and S (officinalis) series of experiments, glass containers were used. For details of the various treatments see the copies of the laboratory sheets attached.

The removal of the liquid was similar to that described above except that the solution was poured through a tin strainer and any

small pieces of bark were returned to the original container. The solution was filtered through paper to remove excess particles of bark, dirt, etc., before precipitation with caustic soda (NaOH).

After the precipitation, the precipitate was filtered off, dried at room temperature, ground to a powder, and assayed for total alkaloids, Quinine plus Cinchonidine, Cinchonine plus Quinidine. Since the original barks contained only traces of Quinidine, estimated to be less than .01%, the Quinidine analyses were made on only a few samples to satisfy ourselves that there was no concentration of Quinidine remaining in the original bark. TCA percentage was obtained by adding the figures representing the separate alkaloid extractions.

No moisture determination was made on the precipitates since the weights of the precipitates were determined and the total alkaloids may be computed from the individual alkaloidal constituents determined by the analyses.

The total alkaloids were determined by the Dutch method which consists of adding an excess of standardized 1.00 N. hydrochloric acid after the extraction, removing the benzol and back-titrating with standard 1.00 N. sodium hydroxide to a pH of $6\frac{1}{2}$ to 7 by using universal pH paper as an outside indicator.

An average molecular weight of 310 was used in the calculations the same as was used in the Dutch method. One of the criticisms of this method is that the amount of alkaloid may be in error plus or minus 1/20 of the amount actually present. However, in considering percentages of alkaloids extracted in each experiment it is found that it makes no difference what molecular weight is assumed since these figures cancel out in the numerator and denominator of the calculation for percentages.

The method of analysis used was essentially that sent out by the F.E.A. on May 16, 1944, and is the one now in use at the laboratories of the Colombian Cinchona Mission. The benzol extraction method is used for the preliminary treatment. The separation of the Quinine and Cinchonidine was not always performed because of the small amounts of tartrates obtained. Except for the initial benzol extraction the method is similar to that used by the F.D.A. in New York City in the analysis of Cinchona bark.

The dried precipitates had to be prepared for extraction in a manner different from the benzol method because that method is intended only for ground barks. The preparation of the precipitate samples was similar to that used at Rutgers University College of Pharmacy. Instead of 16-18 cc of 5% sodium hydroxide for a 20 gram sample, a 5 gram sample with only $2\frac{1}{2}/3$ cc of 5% sodium hydroxide was used. Care has to be taken to see that there are no lumps present before the material is introduced into the thimble. Lack of agreement in the analyses seems to be due to improper preparation of the sample for extraction. Duplicate analyses are reported for the dried precipitates.

SERIES NO.1, BOGOTA							
NO.	MAC. TIME HRS	INDIV. MAC. T HRS	CONC OF ACID % BY VOL	TA ORIG.	TA RESID.	% TA EXTD.	NOTES
1	24	24	H_2O	2.46	2.53*	0	*Some extraneous material extd.
2	84	12	1/2	2.46	1.49	40	
3	84	12	1	2.46	1.48	40	
4	96	24	1/2	2.46	1.76	38	
5	48	24	1	2.46	1.44	41	
6	102	12	1/2	2.46	1.40	43	Mac. No.1=24 hr., No.2= 18hr., Remainder = 12 hr.
7	102	12	1	2.46	1.24	50	Do
8	24	24	H_2O	4.67	4.61	1.4	Water Extraction
9	84	12	1/2	4.67	1.59	66	
10	84	12	1	4.67	1.55	67	
11	48	24	1/2	4.67	1.96	58	Mac. No. 1&4 = H_2O, 2&3 = Acid
12	48	24	1	4.67	2.00	55	Do.
13	90	12	1/2	4.67	1.73	63	Mac. No.1=24 hr., No.2=18hr., Remainder=12 hr.
14	90	12	1	4.67	1.48	68	Do.
15	96	24	1/2	4.67	1.11	76	Circulated @ about 1000 cc/min.

Nos. 1 to 7 Cinchona Pubescens, TA 2.46
8 to 15 Cinchona Officinalis, TA 4.67
Sulphuric Acid Used Throughout.

FIG. 19. TABULAR RECAPITULATION, SERIES NO. 1, BOGOTA

SERIES P, BOGOTA							
NO.	MAC. TIME HRS	INDIV. MAC.T HRS	CONC. OF ACID % BY VOL.	TA ORIG.	TA RESID.	% TA EXTD	NOTES
1	24	24	1/2	2.34	1.74	26	1 KG. Fresh Bark
2	48	24	1/2	2.34	1.43	39	Do.
3	72	24	1/2	2.34	1.33	46	Do.
4	96	24	1/2	2.34	1.12	55	Do.
5	96	24	1/2	2.34	1.21	51	Do.
6	96	24	1/2	2.34	1.09	53	2 KG. Fresh Bark
SERIES S, BOGOTA							
1	24	24	1/2	5.43	4.01	26	1 1/2 KG Fresh Bark
2	48	24	1/2	5.43	2.90	49	Do.
3	72	24	1/2	5.43	2.40	59	Do.
4	96	24	1/2	5.43	1.95	67	Do.
5	96	24	1/2	5.43	2.53	56	Do.

P Series, Cinchona Pubescens, TA 2.34
S Series, Cinchona Officinalis, TA 5.43
Sulphuric Acid Used Throughout.

FIG. 20. TABULAR RECAPITULATION, SERIES P AND SERIES S, BOGOTA

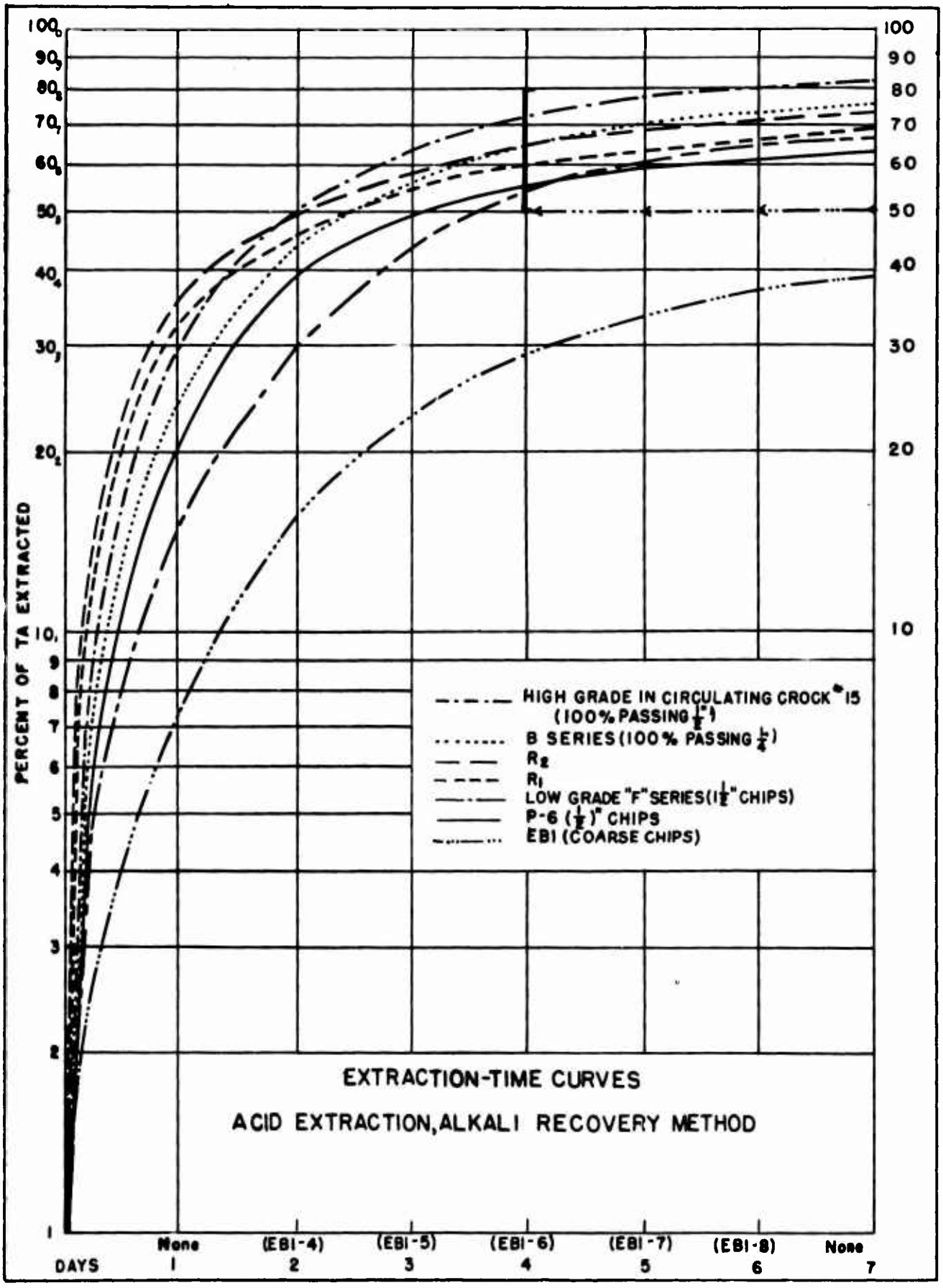


FIG. 21. EXTRACTION-TIME CURVES

C O P Y

COLOMBIAN CINCHONA REGIONS

67, 68, 69
C. pubescens:- Alkaloidal content ranging ½% to about 4%. Average about 1½ to 2%. Area is capable of producing enormous quantities of low grade bark, has never been in production. Large areas of this region are accessible by road or short pack trips.

46 & 47. Antioquia
C. pubescens in great quantities. Alkaloidal content averages about 1%, max. about 2.2%
C. officinalis also of very low alkaloidal content, average about the same as pubescens. Quinine content of officinalis not determined. Much of this area is accessible either by motor road or rail.

48, 49, 50 & 51
C. pubescens: again in great quantity and much of it fairly accessible.
C. officinalis & Remijia are found in this region but discovery recent and not much information available.

2 & 3
C. pubescens: Alkaloidal contents range from 1 to 3.8%, average about 2%. No production and little information concerning transportation facilities.

4 Ocana region
C. officinalis: Best quality of this species found in country but in no great quantity. TCA, 5-6% with large proportion of quinine, also quinidine. Not a good "totaquina bark" because of quinidine. A small quantity of C. officinalis has been harvested in this region.
C. pubescens: TCA ½% to 2.8% average 1.5-%.
Ladenbergia hookeriana: TCA 1% or less, mostly cinchonine. This species was harvested and mixed with C. pubescens, about 600 tons. This bark is now in storage. Mixture mostly Ladenbergia (Quina morada) so TCA is around 1%. The region is quite accessible.

5 & 6
C. pubescens: 40 to 50 tons of bark similar to region 4 have been harvested, shipped through connection with Magdalena R.
C. officinalis: very high yield, TCA 2.8% low, generally 4% to 7.6%, as high as 9%, 3 to 7% quinine sulphate. There is probably a considerable quantity of the species available. Some has been harvested in South end of area. Region is fairly accessible, two day mule trip from motor road. Officinalis here high in quinidine.

60 La Cruz
C. pitayensis: Same quality as above. Has been cut back until fairly inaccessible. About 30 tons harvested.
C. pubescens: Quite variable, 1% to 2½% in considerable quantity, has not been cut.
Here also is found a poor strain of officinalis.

61 to 64 Pasto
Same species as above. Quite a lot, up to 100 tons of pitayensis has been taken out and is becoming quite inaccessible.

70, 71, 72
Not sufficient exploration. Pitayensis is known to exist in area, has not yet been harvested. There is also a great deal of poor pubescens, 1½%. Pitayensis is inaccessible, country very wet.

73 & 74 San Pablo, Argelia
C. pitayensis: Same quality as above. A large amount has been harvested but much remains. Very rough country. Also much pubescens.

75 to 78 inc.
C. pitayensis: A great deal of species has been harvested and there is still much remaining in less accessible areas. Enormous quantities of pubescens, some of which runs as high as 2½%.

79 Mayasquer, Cumbal
C. pitayensis: A large quantity has been harvested. An unknown variety of pitayensis has been reported in lower elevations of region. TCA of this variety seems about same as regular species. There are two forms of pubescens, one of which runs 2½% all cinchonine. Pitayensis has been cut from all accessible areas. Lower elevation variety will be very difficult to get to.

General
Region 20 may prove good place to start acid extraction since even high grade bark cannot be harvested at profit because of transportation difficulties. Remijia around Velez in region 11 presents opportunity to try method on low yield, high quinine bark.
Pitayensis - Argelia, San Pablo, region 73. Very difficult to reach, two day pack trip, presents best possibilities for this species.

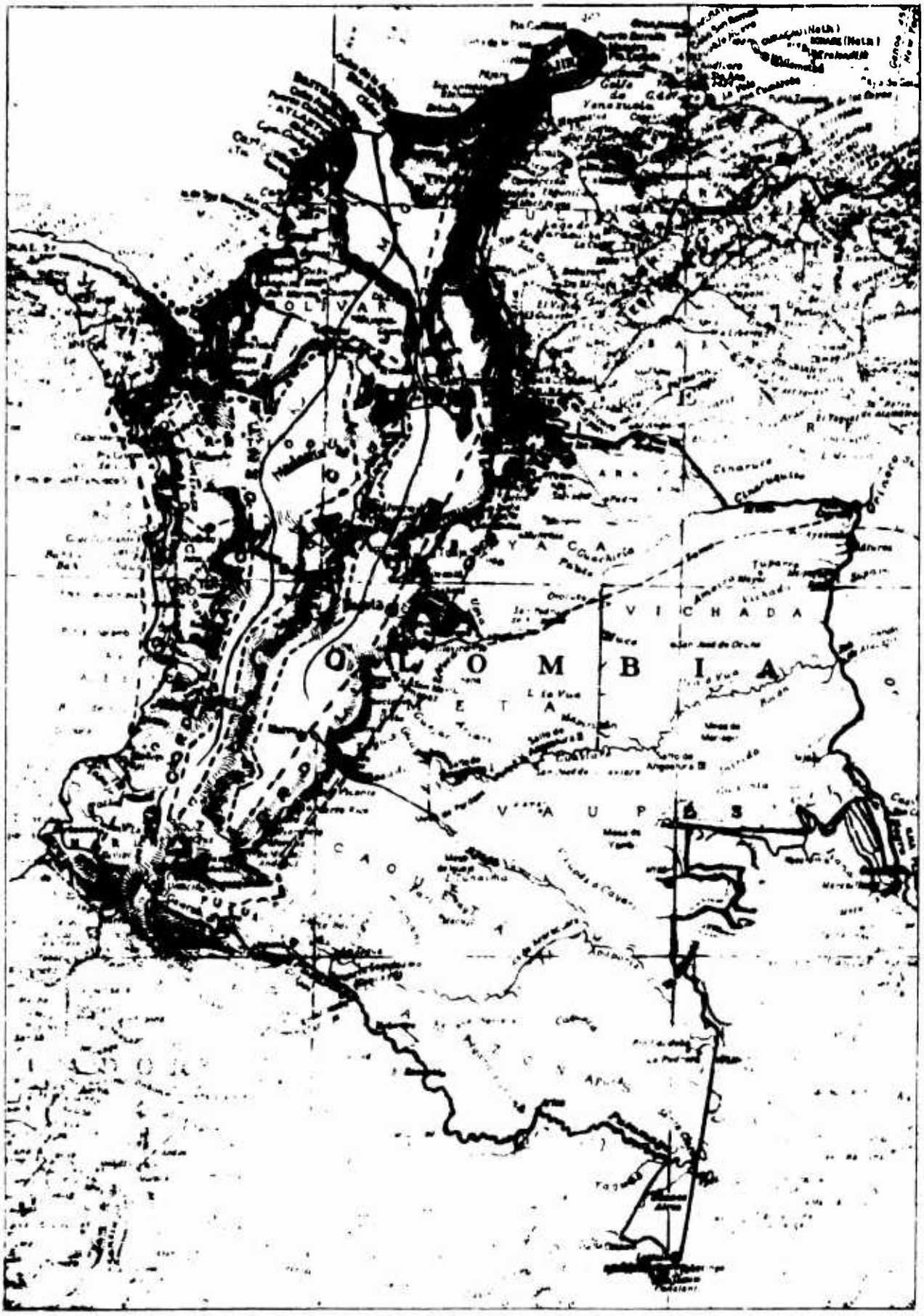


FIG. 22. MAP, COLOMBIAN CINCHONA REGIONS

AMERICAN EMBASSY
Office of the Military Attache
Lima, Peru

15 June 1944

MEMORANDUM TO CAPTAIN KAYE:

Subject: Productive Capacity Peruvian Quina Districts as Given by Mr. Fox, Cinchona Mission, Lima, Peru.

1. Tambopata Valley.

Production 1943 - 13 tons bark mixed, 1944, 100 tons high grade. (C. calisaya and mirada, 2.5 - 6% Quinine sulphate, which generally constitutes about 65 - 75% of T.C.A.)

This area with existing animals working only on Quina might be capable of harvesting and transporting to nearest truck road 150 tons of bark. With transportation factor removed, production could be doubled; further expansion would require importing labor.

Mr. Fox suggests this valley as plant site for high grade bark. This involves four day mule trip and three day pack. Engineer reconnaissance now being conducted to extend mule trail and develop airfields. Airfield would be quite close to source of bark.

There is also much low grade (cinchonine 2%, cinchonidine 1-½% approximately) bark, C. micrantha in this area.

All the present production goes to Sociadad Nacional de Quina factory in Lima. If bark were available, plant could increase production to 200 tons per year.

2. Huari Huari

Transportation problems similar to Tambopata; bark is somewhat lower in quinine than that of Tambopata. Produces 100 tons per year, could be stepped up to 400.

3. Cosnipato.

Low grade (2%) bark, C. micrantha not being exploited at present. Cinchonine-cinchonidine with traces of quinine.

4. Convension (Cuzco)

Fairly accessible (one day, mule train from road) C. officinalis, T.C.A. 3-½% Quinine sulphate .3% to .5%, Cinchonine 2.5%, cinchonidine 1%. May be a good totaquina bark. 150 tons per year.

5. Tingo Maria.

C. pubescens, TCA 3½%, almost all cinchonine. Bark supply accessible from road. At Tingo Maria there is an agricultural station, building facilities only. Area can produce 200 to 300 tons per year. Transportation no factor.

6. Satipo, Tingo Maria, Chunchas Mayo.

About same in all respects.

7. Apurimac.

No production at present, no plans. Same bark as Convension. Probably could produce 50 to 100 tons.

8. Uchiza.

Same as Tingo Maria save for transportation problem which is difficult.

9. Pomacocha.

2½% cinchonine bark. Possible 400-ton production. Airfield, dryer and warehouse built.

10. San Ignacio.

Same as Bagua.

11. Penachi.

Very accessible; one day by mule. Mainly cinchonidine; some cinchonine, traces of quinine. Totaquina bark. 200 tons per year now being produced, could not be increased much. Species being harvested as C. officinalis.

12. Summary:

"The entire country is now producing approximately 1500 tons of dry bark per year under the present transportation situation. If acid extraction or the development of transportation facilities, airfields, mule trails, etc., were applied to break the transportation bottle-neck, production would be easily doubled. Subsidizing of transportation agency has been considered in this respect. Field extraction would be the cheapest and most effective means of solving the transportation problem and would in all probability increase production to a greater extent." (Quoted from a conversation with Mr. Fox)

/S/ Silvio E. Ronzone
SILVIO E. RONZONE.
2nd. Lt., Corps of Engineers.

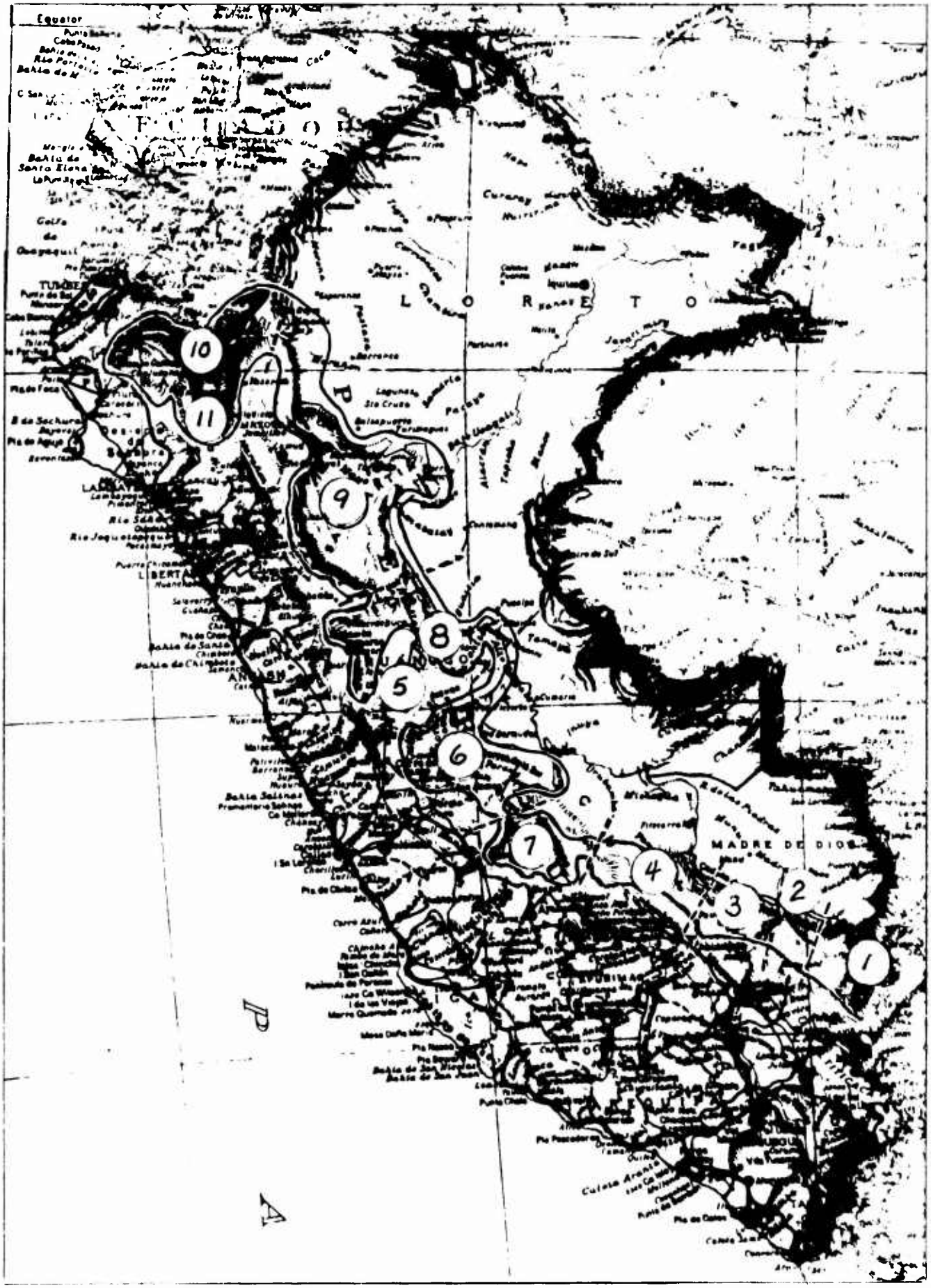


FIG. 23. MAP, PERUVIAN CINCHONA REGIONS

MEMORANDUM

TO: Captain Kaye

FROM: Lt. Ronzone

SUBJECT: Cinchona Producing Areas of Ecuador

AREA NO. I.

Cinchona pitayensis: Western slope of Cordillera Occidental from the Colombian border to Quito. Exploration is now being conducted on eastern slopes. This species occurs at 9,000 - 10,000 ft. elevation. Average TCA is 5% (4.5-6.5%) average quinine sulphate 2.5%, quinidine 2.5%.

Area now produces about 60 tons of dry bark per year (based on seven months of operation). The chief limiting factor in this area is that the extremely wet climate makes it impossible to dry bark on the spot. Approximately one-half the bark produced by this area is now being transported a distance of from ten to twenty miles to a desert area where drying is possible. If the drying factor were eliminated production could be doubled. Existing supplies would probably be exhausted in two to three years under intensive exploitation. The area is accessible by one day horse or pack trip from the nearest truck road. Additional supplies will probably be revealed by exploration now underway.

AREA NO. II. (El Topo - Rio Pastaza)

Cinchona officinalis: Eastern slope of Cordillera Oriental extending twenty-five miles north and twenty-five miles south of the Rio Pastaza at 4,000 - 5,000 ft. elevation. Average TCA 4%, quinine sulphate 1 - 1.5%, cinchonidine generally higher than cinchonine.

This area is now producing about 240 tons of dry bark per year. It would be a good area for field extraction since transportation and drying are the limiting factors. If these factors were eliminated the production could be doubled or more.

Field extraction would release about two-thirds of the existing labor supply which is now engaged in packing green bark to dryers, at present a distance of about ten miles. This area will last for two or three years under intensive exploitation. New supplies are being uncovered by exploration.

AREA NO. III

Cinchona pubescens: Western slope of Cordillera Occidental, Provinces of Bolivar and El Oro, extending 100 miles south of Guaranda to about Zaruma at 4,000 - 7,000 ft. elevation. Average TCA 3-5% nearly pure cinchonine.

Area has produced 700 tons of dry bark per year (during May, 122 tons). Is now operating at this capacity (3% bark) though the product contains no quinine.

There is a sub-area of this region, between 2,000 - 4,000 ft. elevation where a special race of pubescens called "roja" occurs in plantations, which produces a bark yielding 1.5% - 2% quinine and 4% TCA. This locality is now being exploited at about its maximum sustained yield capacity. Transportation is not a major problem, the area being accessible generally by road and trail. It produces about 100 tons per year.

This would be an excellent location for a semi-permanent extraction plant such as the type planned for El Porvenir.

AREA NO. IV

Cinchona officinalis: Cordillera Oriental, provinces of Azuay and Loja about 100 miles in length between the elevations of 5,000 and 7,000 feet. In general this region yields a low grade bark with about 0.5% quinine sulphate and 3% TCA. The primary use of this bark is in the manufacture of totaquina locally, During the past year about 50 tons was produced. The region is reasonably accessible but here again production is limited by transportation and drying factors. Bark could be produced at the present rate for an indefinite period. Most of the present production is sent to Zaruma, Quito, and Guayaquil for extraction. A totaquina plant at Loja has temporarily suspended operation since the owners are on the "Black List". A higher yielding strain called "Urituzinga" is found in isolated patches (2% quinine and 4% TCA) but it has been exploited almost to extinction.

GENERAL.

If alkaloids containing mostly cinchonine were desired, a low yielding race of pubescens (1 - 1.5% TCA with traces of quinine) occurs in abundance in all parts of both mountain ranges.

Present production of 700 tons for the entire country could be increased to 1,000 tons per year by the installation of field extraction units, according to Dr. Steere.

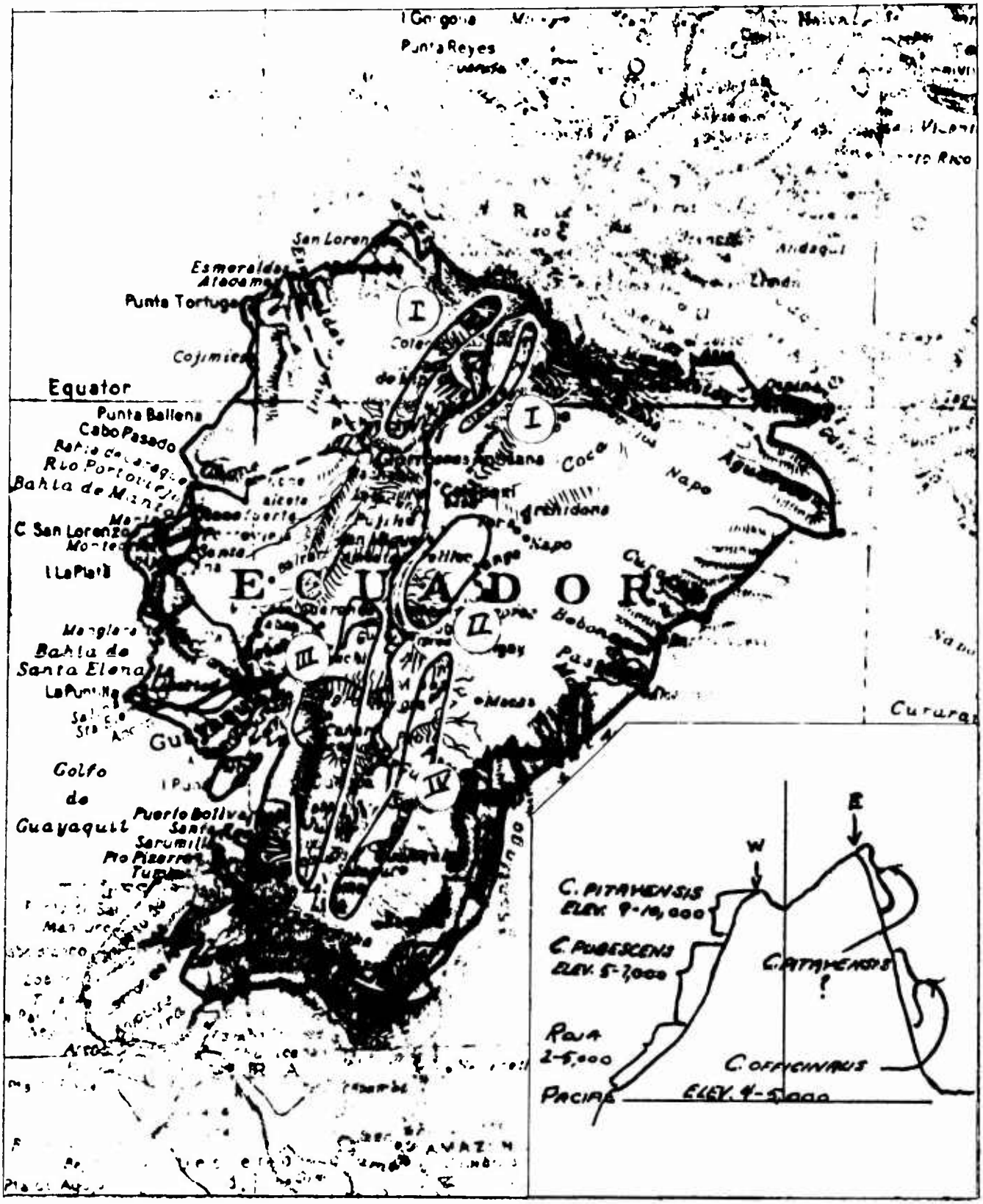


FIG. 24. MAP, ECUADORIAN CINCHONA REGIONS

APPENDIX C

ENGINEER BOARD EXPERIMENTS

Illustrations

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 1

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 15 Nov 44

Pounds of Bark	16.7	Total Solids Recovered	
Size of Grind	Chips	TA of Recvd Solids	
TA Original	5.79%	% TA Extd. & Recovered	Recovery not
TA Residual	2.84%	% TA Orig. Recovered	Attempted
% TA Extracted	51		

OPERATIONS DATA			
Maceration Time	23 Hr.	Conc. & Vol. of Menstruum H_2SO_4	.1 N, 50 l.
Stirring Time	0 "	Conc. & Vol. Alkali Regen./Col.	5%, 24 l.
Circulation Time	13 3/4"	Conc. & Vol. Acid. Regen./Col.	.5 N, 12 l.
No. Fluid Cycles	—	Rate of Application of Regen.	< 300 cc/min.
No. Columns	2	Elutriant, Vol.	108 l.
Total Bed Volume	9 l.	Application of Elutriant	Continuous Flow

NOTES

Apparatus: Rubber slurry bag with percolator made from cotton flour bag, assembled for downflow at rate of 1000 cc/min. Five 4.5 l. ion exchange columns were improvised from 5 gpm filter shells.

Extraction: Flow rate at start of experiment varied from 300 cc/min. to as high as 4000 cc/min. in effort to find optimal rate.

Bark, prepared by presoaking, consisted of medium chips of Guat. 509. Fifty liters of .1 N H_2SO_4 were used for this maceration.

Circulation continued for 5 hours, was shut down for the night, and was resumed for 8 3/4 hours on following day.

Recovery: During this experiment, alkali regeneration by means of Na_2CO_3 was attempted. Though it seems possible to obtain adequate regeneration by this means, the effusion of CO_2 was so great as to force the ZeoKarb through the connections to the column. The use of the carbonate thus appears to be impractical.

Following alkali regeneration, 108 liters of ethyl alcohol were run through the two columns used at a rate of 100 cc/min. before an approach to negative Mayers could be reached. The alcohol wash was followed by a 10 liter water wash, 5 liters of which were saved for distillation with the alcohol.

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 1

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 15 Nov 44 CONTINUED:

Recovery: Distillation of the elutriant produced about 12 liters of dark red concentrate. Efforts were made to recover the alkaloids from this liquid by filtering with diatomite, inducing flocculation with ammonia alum, and increasing the pH, which was naturally about 8-9 up to 12. No satisfactory results were obtainable, so various portions of the liquid concentrate were reduced to dryness over a water bath. These lots introduced variations in pH, attempts to induce crystalization through reduction in volume, and temperature reduction. None of the above was attended by any degree of success, so it seems necessary to accept the fact that these concentrates will have a definitely syrupy consistency and attempt final concentration by evaporating to dryness.

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 2

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 17 Nov 44

Pounds of Bark	16.7	Total Solids Recovered	
Size of Grind	Chips	TA of Recvd Solids	
TA Original	5.79%	% TA Extd. & Recovered	Recovery not
TA Residual	1.94%	% TA Orig. Recovered	Attempted
% TA Extracted	65		

OPERATIONS DATA			
Maceration Time	76 Hr.	Conc. & Vol. of Menstruum	.1 N, 50 l.
Stirring Time	0 "	Conc. & Vol. Alkali Regen./Col.	.5 N, 12 l.
Circulation Time	33 1/6"	Conc. & Vol. Acid. Regen./Col.	.5 N, 12 l.
No. Fluid Cycles	40	Rate of Application of Regen.	Cycled
No. Columns	2	Elutriant, Vol.	75 l.
Total Bed Volume	9 l.	Application of Elutriant	Cycled

NOTES

Apparatus: Small fabric tank assembled with fabric percolator having aluminum bottom, downflow at rates varying from 500 cc/min. to 2000 cc/min. was employed.

Two filter shell cylinders and a fraction of a third were used for the ion exchange system in this experiment.

Extraction: Flow rates were varied in attempt to find optimal for system. Influent became positive through early stages of the extraction at rates above 800 cc/min. By end of run influent remained negative at rates up to 1200 cc/min.

The effluent remained positive throughout the test, becoming very faintly so after 76 hours.

Recovery: Prior to alkali regeneration the cylinders of the ion exchange system were washed by upflow of water for several hours before a pH of 6+ was reached. Following the water wash, 24 l. of .5 N NaOH were recirculated through the beds at a slow rate. The regeneration was followed by another water wash until pH was reduced to 7+.

Twenty liters of alcohol were cycled through the beds for 2 1/2 hours, this quantity was displaced with fresh alcohol which was in turn displaced by 15 liters more of fresh alcohol, with a Mayer's test taken at each liter, flow rate at 100 cc/min. The 15 liters were then recirculated

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 2

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 17 Nov 44 CONTINUED:

Recovery: at 1400 cc/min. for 7 hours, another 16 liters of fresh alcohol introduced, a Mayer's test still showed positive so 20 more liters were put through, followed by still another 20 liters at the rate of 100 cc/min. By this time the effluent alcohol though still positive was weak and erratic with Mayer's reagent. Some 100 liters in all were used to reach this point.

A brick red concentrate resulted from distillation of the alcohol. Further attempts were made to remove the solids from this fluid by filtration with diatomite, reduction of volume by evaporation followed by filtration and centrifuging, but without success.

A portion of the concentrate was evaporated to dryness and saved for further treatment.

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 3

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 22 Nov 44

Pounds of Bark	16.7	Total Solids Recovered	111.89
Size of Grind	Chips	TA of Recvd Solids	—
TA Original	5.79%	% TA Extd. & Recovered	13.6
TA Residual	1.13%	% TA Orig. Recovered	11.1
% TA Extracted	80.4	% TA $CHCl_3$ Concentrate	66.5

OPERATIONS DATA			
Maceration Time	64 3/4 hrs.	Conc. & Vol. of Menstruum	.1 N, 18 l.
Stirring Time	0	Conc. & Vol. Alkali Regen./Col.	.5 N, 7 l.
Circulation Time	23 "	Conc. & Vol. Acid. Regen./Col.	.5 N, 14 l.
No. Fluid Cycles	—	Rate of Application of Regen.	600 cc/min
No. Columns	3	Elutriant, Vol.	62 l.
Total Bed Volume	13.5 l.	Application of Elutriant	Cycled

NOTES

Apparatus: Maceration equipment assembled as in EB2-2.

Ion exchange system consisted of three 4.5 liter beds arranged to operate counter-currently upflow.

Extraction: Circulation started at very fast rate, 2600 cc/min. at once became strongly positive. Successive reductions in rate to 100 cc/min. before a negative was reached. At 14 hours of circulation the flow rate was increased to 1000 cc/min. before breakthrough was reached. By the end of 23 hours circulation time, it was not possible to observe any decrease in alkaloid concentration between influent and effluent. Experiment terminated.

Recovery: Alkali regenerant consisting of 7 l./col.of .5 N NaOH was run through system at very slow rate, columns having been reduced to pH 6+ by continuous water wash for two hours. Alkali regeneration was followed by continuous water wash for about six hours before pH of columns could be brought to a point below 8.

Elutriation was accomplished by recirculating 54 liters of alcohol in three separate lots for a total period of 10½ hours. The third lot was recovered in two batches, the latter containing considerable water as alcohol had been displaced by upflow of H_2O.

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- _3_

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 22 May 44 CONTINUED:

Recovery:

Lots 1 and 2 were combined for distillation, as were lots 3 and 4. The concentrate, which was dark red in color, was reduced to dryness in a constant temperature oven. Lots 1 and 2 produced a total of 96.1 grams of dry material; lots 3 and 4 produced only 15.79 grams.

The 111.89 grams of dry concentrate were divided into five Soxhlet extractors and extracted with $CHCl_3$ for a total extraction time of some 36 hours. At the end of this time a positive Mayer's resulted from a test on the chloroform in the extraction chamber. This was probably due to the fact that the material had packed down in the bottom of the thimbles in such a manner as to be very difficult to extract.

Distillation of the chloroform, followed by oven drying, yielded 68.3 grams of buff colored concentrate which was assayed at 66.5% TA. This represents a recovery of only 13.6% of the amount of alkaloid extracted.

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 4

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 5 Dec 44

Pounds of Bark	17.0	Total Solids Recovered	359.88
Size of Grind	½" Chips	TA of Recvd Solids	78.71%
TA Original	7.45%	% TA Extd. & Recovered	76.00
TA Residual	2.60%	% TA Orig. Recovered	50.00
% TA Extracted	65.1		

OPERATIONS DATA			
Maceration Time	83 1/4 Hr.	Conc. & Vol. of Menstruum	.1 N, 20 l
Stirring Time	0	Conc. & Vol. Alkali Regen./Col.	
Circulation Time	72 1/4 "	Conc. & Vol. Acid. Regen./Col.	.5 N, 12 l
No. Fluid Cycles	—	Rate of Application of Regen.	600 cc/min
No. Columns	5	Elutriant, Vol.	75 l.
Total Bed Volume	27.5 l.	Application of Elutriant	Cycled 3 lots

NOTES

Apparatus: Equipment assembled as in EB2-2. Small disks of screen had been inserted at top outlets of ion exchange cylinders to prevent carrying over of ZeoKarb. These had to be removed as back pressure developed at once. A large line filter was also used to trap fines being carried over from the tank.

Extraction: This experiment was intended to produce some quantitative results, and as such was run continuously for about 75 hours.

It is probable that the volume of the menstruum in relation to the moisture content of the bark (which was presoaked for 24 hours) was such that the acid concentration was insufficient. Extraction rate seemed very slow.

Flow rates of 1000 to 1300 cc/min. were maintained throughout the experiment. Influent tests were consistently positive until some 40 hours of circulation. At this time a freshly regenerated column was put into the system. Thereafter, rates up to 1300 cc/min. remained negative.

At the end of 83 1/4 hours the liquid in the macerator was still positive to Mayer's. The ion exchange system having been fully loaded, the experiment was terminated.

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 4

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 5 Dec 44 CONTINUED:

Recovery: Due to the fact that influent tests were positive from the start, it was decided that an arbitrary figure of 8 hours would be taken as the time required for loading an ion exchange bed. This procedure was followed as nearly as was possible. However, regeneration and stripping were very slow due to attempts to wash the pH back to neutral.

Alkali regenerant quantity was reduced to about ½ the amount used in previous experiments, 7 l./col. of .5 N NaOH being used instead of the 12 liter or "double regeneration".

Alcohol stripping was accomplished by cycling 8 l. of alcohol through each cylinder for 2 hours, followed by another 8 l. cycled for 1 hour.

Evaporating the alcohol concentrates to dryness produced 359.88 grams of material having a TA% of 78.71. It was noted here that the solids recovered from watery concentrates are lighter in color, easier to handle and have a semi-crystalline appearance as compared to the dark amorphous concentrates from strongly alcoholic concentrates.

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 5

Date 26 Dec 44 ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Pounds of Bark	17.0	Total Solids Recovered	193.51
Size of Grind	Chips > ½"	TA of Recvd Solids	74.69%
TA Original	6.89%	% TA Extd. & Recovered	56
TA Residual	3.49%	% TA Orig. Recovered	27
% TA Extracted	49		

OPERATIONS DATA			
Maceration Time	34 3/4 Hr.	Conc. & Vol. of Menstruum	.1 N, 45 l.
Stirring Time	0	Conc. & Vol. Alkali Regen./Col.	.5 N, 12 l.
Circulation Time	33 1/4 "	Conc. & Vol. Acid. Regen./Col.	.5 N, 12 l.
No. Fluid Cycles	—	Rate of Application of Regen.	200 cc/min.
No. Columns	6	Elutriant, Vol.	60 l.
Total Bed Volume	27.0 l.	Application of Elutriant	Cycled 3 lots

NOTES

Apparatus: Apparatus assembled for upflow circulation through three columns at once. At end, four columns were used. Experiment first tried with fine bark, but downflow in macerator caused plugging, so fine bark had to be abandoned. Chips larger than ½ inch were used.

Extraction: Here it was found for the first time that the adsorption rate overtakes the extraction rate as the latter diminishes. It was noted that after 21 hours of circulation a negative influent was obtained at a flow rate of 2500 cc/min. However, with the maceration standing for two hours, the fluid in the macerator became positive to the extent that it broke through at 2000 cc/min. This is probably the explanation of how the Rutgers laboratory observed a negative after only 24 hours of circulation. Experiment was continuous.

Recovery: Though the alkali regenerant was supposedly applied according to the method producing the best results to date (12 liters .5 N at 200 cc/min.) considerable trouble was experienced in getting satisfactory results from the ion exchange system. Columns 1 and 4 were regenerated during the run. No. 4 seemed to function poorly when replaced, while No. 1 did not appear to have been regenerated at all (almost no solids were recovered from this stripping of No. 1). At the end of the run four columns were regenerated. This was accomplished by percolating 24 liters of .5 N NaOH through two columns in tandem. This was followed by cycling 60 liters of alcohol through the entire system in three

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 5

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 26 Dec 44 CONTINUED:

Recovery: 20 liter lots, each lot being circulated for 1 hour. Lots 2 and 3 were combined for distillation, lot 1 being distilled separately.

109.82 grams of solids were recovered from evaporation of lot 1 concentrate. Lots 2 and 3 yielded 83.69 grams for a total of 193.51 grams of material having an average TA% of 74.69.

The concentrate from lot 1 was a light pink, very fluffy material, that from lots 2 and 3 was dark red, denser though definitely a crystalline material.

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 6

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 3 Jan 45

Pounds of Bark	10.0	Total Solids Recovered	204.67
Size of Grind	< ½ inch	TA of Recvd Solids	16.29%
TA Original	4.65%	% TA Extd. & Recovered	21.00
TA Residual	1.39%	% TA Orig. Recovered	16.00
% TA Extracted	72.00		

OPERATIONS DATA			
Maceration Time	72½ Hr.	Conc. & Vol. of Menstruum	.1 N, 54 l.
Stirring Time	0	Conc. & Vol. Alkali Regen./Col.	.5 N, 15 l.
Circulation Time	21 "	Conc. & Vol. Acid. Regen./Col.	.5 N, 12 l.
No. Fluid Cycles	24	Rate of Application of Regen.	<300 cc/min.
No. Columns	3	Elutriant, Vol.	
Total Bed Volume	13.5	Application of Elutriant	

NOTES

Apparatus: This experiment was operated upflow throughout because of the presence of a considerable amount of fines. A simple flow meter, consisting of a 2½-inch lucite cylinder calibrated to measure flow rate by length of stream entering the cylinder through a small jet, was installed and found to work as well either on suction in a closed system or by gravity at the outfall. Back pressure continuously developed during this experiment, and all attempts to alleviate the condition proved futile.

Extraction: Bark was soaked for about 4 hours in H_2O. This fluid was made up to .1 N with H_2SO_4 and cycled through the macerator for about four hours more. Three ion exchange columns were then cut into the system and circulation continued. Throughout the experiment negative influents were maintained with flow rates varying from 800 to 1500 cc/min. After 72½ hours of operation during which circulation was carried on for 21 hours, the system became so plugged that further operation proved impossible.

Recovery: Alkali regeneration was attempted by introducing three separate lots of .5 N NH_4OH of 5 liters to each column. The first of these was for the purpose of neutralizing the acid beds. It was noted here that in neutralizing column No. 2, which had been in the "sweep up" position, almost the entire first five liters were required before a pH of 7 was reached. Column No. 1, second in line, required about 2 liters, while No. 4, which was first in line, required 3½ liters to produce the same change.

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 6

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 3 Jan 45 CONTINUED:

Recovery: Three liters of alcohol (amount required to fill voids in a 4.5 l. bed) were introduced to each column and circulated downflow for one hour. This was followed by 3 liters of fresh alcohol circulated for two hours. Following this 2 liters more alcohol were introduced downflow and the beds drained down. Beds then washed by upflow of water, the first liter being saved for concentration.

Distillation of the alcohol showed the corrosive effects of ammonia on the copper parts of the still and circulation system. The recovered alcohol was very green in color and smelled strongly of ammonia.

A total of 100.58 grams of solids was recovered from evaporation of the concentrate. Since the water left in the ion exchange beds was highly colored, it was decided that this water should be concentrated. It produced 94.31 grams of dark red solid. A further wash with 17 liters of alcohol yielded 8.78 grams. The total of these lots, 103.99 grams was combined for assay.

Cleaning up the alcohol proved to be a difficult task, several distillations being required.

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 7

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 22 Jan 45

Pounds of Bark	17.5	Total Solids Recovered	
Size of Grind	40 Mesh	TA of Recvd Solids	
TA Original	4.96%	% TA Extd. & Recovered	Recovery not
TA Residual	2.39%	% TA Orig. Recovered	Attempted
% TA Extracted	52.00		

OPERATIONS DATA			
Maceration Time	99.0 Hr.	Conc. & Vol. of Menstruum	.1 N, 60 l.
Stirring Time	0 "	Conc. & Vol. Alkali Regen./Col.	See Notes
Circulation Time	31.5 "	Conc. & Vol. Acid. Regen./Col.	.5 N, 12 l.
No. Fluid Cycles	31.5 "	Rate of Application of Regen.	See Notes
No. Columns	4	Elutriant, Vol.	24 l.
Total Bed Volume	18 l.	Application of Elutriant	Cycled 2½ Hr.

NOTES

Apparatus: This was the first attempt to use 40 mesh bark in the scale model operation. As in EB2-6, it was necessary to use upflow circulation through the macerator. In the preceding experiment a great deal of time was lost in trying to find the cause of back pressure in the system. This pressure, which developed with any protracted period of circulation, would gradually decrease the flow rate until circulation stopped entirely.

It was thought that the cause of the back pressure might be a tendency on the part of the ZeoKarb mass to rise in a solid body to a point where it cut off the top outlet of the column. This theory was later found to be true, at least in part. The rising of the entire mass seems to be due to a collection of air at the bottom of the column forming a large bubble which pushes the zeolite slug ahead of it.

Apparatus was installed to reverse the direction of flow through the entire ion exchange system. This expedient was only partially successful in alleviating the difficulty. However, it did destroy the countercurrent effect of the operation, and so is not considered a usable addition to the process. (Later experiments proved that most back pressure resulted from accumulating ZeoKarb in the hose line, valves, and bottom screens of the columns. This material was carried over by surging flow from the preceding column.)

Extraction: With the apparatus assembled it was found very difficult to obtain any sort of consistent continuous operation. No adequate line filter was used, so a great deal of the colloidal fines was carried over in the menstruum. This material in suspension made Mayer's determinations difficult and erratic. Constant variation in flow rate made the computation of circulation rate most unreliable.

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 7

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 22 Jan 45 CONTINUED:

Recovery: Regeneration of the ion exchange system was attempted with Na_2CO_3. It was reasoned that the effusion of CO_2 which had made previous attempts to use this chemical unfeasible could be induced outside the ion exchange columns. The procedure followed made use of the well known difficulty of dissolving this carbonate. 750 grams of Na_2CO_3 were suspended in a cotton sack over a rubber bag. The fluid from the ion exchange system was circulated through the bag, draining through the carbonate which was in briquette form, in the process. It was found, as expected, that the greater part of the effervescence occurred in the rubber bag. Such gas effusion as took place in the columns was not sufficient to cause any difficulty. The dissolving of the 750 grams of carbonate raised the pH of the system from 1 to 8, but was not successful in releasing the alkaloid since concentration of a one liter aliquot of the elutriant produced only a negligable recovery.

Why the pH of 8 should have been reached without release of the alkaloid is not understood. Obviously only the unused portion of the bed was regenerated plus neutralization of the excess acid solution.

CINCHONIA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 8

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 30 Jan 45

Pounds of Bark	10	Total Solids Recovered	116.20
Size of Grind	¼" Hammer mill	TA of Recvd Solids	41.90%
TA Original	2.88%	% TA Extd. & Recovered	45.55
TA Residual	0.35%	% TA Orig. Recovered	40.07
% TA Extracted	88.		

OPERATIONS DATA			
Maceration Time	72 Hr.	Conc. & Vol. of Menstruum	.1N, 60 l.
Stirring Time	45½ "	Conc. & Vol. Alkali Regen./Col.	.5N, 12 l.
Circulation Time	27 "	Conc. & Vol. Acid. Regen./Col.	.5N, 12 l.
No. Fluid Cycles	17.1	Rate of Application of Regen.	<300 cc/min.
No. Columns	3	Elutriant, Vol./Col.	14 l.
Total Bed Volume	13.5 l.	Application of Elutriant	Cycled 2 Hr.

NOTES

Apparatus: A three cubic feet monel metal filter shell was used as maceration tank for this experiment. This tank was assembled for upflow operation as it was expected that downflow through finely ground bark would not be possible. At the end of the experiment the entire mass of bark was removed from the macerator, dried, reground to 40 mesh, and a sample for assay taken by successive quartering.

Extraction: Circulation in this experiment was run until a negative or nearly negative effluent resulted. Stirring was resumed as soon as it was decided that a circulation period had progressed far enough and continued until one hour before circulation was to be resumed. Flow rate was held at 1000 cc/min. throughout. However, trouble was experienced in preventing clogging of the intake strainer at this rate. An attempt was made to stir and circulate at the same time but without success.

Recovery: Each column was regenerated individually by the following means. Column first washed by upflow of water at 1000 cc/min for 15 minutes. This reduced the pH to about 4, after which 12 liters of .5 N NaOH were passed through at <300 cc/min. The alkali was followed by 15 liters of water upflow at <300 cc/min. Columns were then drained and blown down, three liters of methyl alcohol introduced upflow, allowed to stand for 10-15 minutes, and drained down. Eight liters of methyl alcohol were then circulated for two hours, columns drained and blown down, 3 liters fresh alcohol introduced upflow, displaced by upflow of water, 4 liters being collected at top of column. Forty-eight liters of alcohol and water were reduced by distillation to 9 liters which were reduced to dryness, yielding 116.20 grams of solids having a TA of 41.9%.

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 8A

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 7 Feb 45

Pounds of Bark	10	Total Solids Recovered	309
Size of Grind	¼" Hammer mill	TA of Recvd Solids	49.7%
TA Original	5.56%	% TA Extd. & Recovered	100*
TA Residual	—	% TA Orig. Recovered	65
% TA Extracted	65*		

*Based on actual recovery

OPERATIONS DATA			
Maceration Time	76 Hr.	Conc. & Vol. of Menstruum	.1 N, 60 l.
Stirring Time	8 "	Conc. & Vol. Alkali Regen./Col.	.5 N, 12 l.
Circulation Time	20 "	Conc. & Vol. Acid. Regen./Col.	.5 N, 12 l.
No. Fluid Cycles	8.6	Rate of Application of Regen.	300 cc/min.
No. Columns	4	Elutriant, Vol.	14 l.
Total Bed Volume	18 l.	Application of Elutriant	

NOTES

Apparatus: Macerator assembled for upflow, a single thickness of osnaburg cloth was stretched over the perforated metal percolator bottom. An electrically powered mixer was mounted over the macerator and operated at a speed sufficient to produce thorough agitation for a period of 8 hours. Circulation was then started through a system of three ion exchange beds.

Extraction: Flow rate was originally set at 1000 cc/min. but as influent became positive at once, the rate was cut to 500 cc/min. The influent continuing positive, column No. 4 was introduced. This reduced the alkaloid concentration of the influent considerably but the advantage held for only about one hour. A series of Mayer's tests on dilutions from the macerator and from column No. 4 indicated that the alkaloid concentration in the macerator was 2½ times that of the influent after 8 hours of circulation. By the end of 76 hours total maceration time the alkaloid concentration in the macerator had decreased from 1/4000 (at 32 hours) to 1/17000. The influent to the macerator had remained positive throughout the test.

Bark samples were taken at regular intervals throughout the extraction but proved later to be of no value, as a great portion of the fines (sizes smaller than 40 mesh) had worked through the bottom of the macerator. On this account the extraction figures had to be based on recovery data alone.

Recovery: Alkali regeneration was performed according to a standard method as follows. Each column was washed by rapid upflow of water until pH had been built up from 1 to about 4. At this point 12 l. of .5 N NaOH were introduced upflow, at a rate of less than 300 cc/min.

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 8A

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 7 Feb 45 CONTINUED:

Recovery: The columns were then allowed to stand for 10 minutes. Columns were then drained down and three volumes of H_2O put through at the same rate, the columns being drained and blown down after this wash. Three liters of fresh alcohol were then introduced into each column and allowed to stand for about 10 minutes. Columns were then drained down and 8 liters of alcohol circulated upflow for two hours. The eight liters were drained off and three more liters introduced upflow and displaced by upflow of H_2O, 4 liters being recovered at outlet. The columns were then washed by rapid upflow of water for 10 minutes.

In this experiment three liters of hot alcohol were used in addition to the last alcohol wash, making a total of 17 liters per column. All columns were regenerated and stripped separately through the EB2-8 series.

The alcohol was concentrated in several batches yielding a total of 309 grams of recovered solids with a TA of 49.7%.

In performing the first 3 liter alcohol wash, it was found that the amount of residual water on the ZeoKarb was sufficient to overcome the solubility ratio, and about 18 grams of pure alkaloid was deposited in the effluent bottle. Undoubtedly a similar condition existed within the columns.

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 8B

Date 13 Feb 45 ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Pounds of Bark	10	Total Solids Recovered	305.6
Size of Grind	¼" Hammer mill	TA of Recvd Solids	53.3%
TA Original	5.56%	% TA Extd. & Recovered	100.0
TA Residual	*	% TA Orig. Recovered	76.0
% TA Extracted	64.4*		

*Based on assay and grain size analysis of fines lost.

OPERATIONS DATA			
Maceration Time	76 Hr.	Conc. & Vol. of Menstruum	.1 N, 60 l.
Stirring Time	8 "	Conc. & Vol. Alkali Regen./Col.	.5 N, 12 l.
Circulation Time	20 "	Conc. & Vol. Acid. Regen./Col.	.5 N, 12 l.
No. Fluid Cycles	8.6	Rate of Application of Regen.	300 cc/min.
No. Columns	4	Elutriant, Vol/Col.	14 l.
Total Bed Volume	18.0 l.	Application of Elutriant	Cycled 2 Hr.

NOTES

Apparatus: Equipment assembled as in EB2-8A with the addition of two 5 gpm filter shells with monel wound Stellar elements which were used alternately as line filters to remove bark fines from the effluent. The suction line from the pumpswas equipped with a cloth covered guard to prevent the incursion of bark particles large enough to clog the pump.

Extraction: Circulation was started using three ion exchange beds. The influent became positive at once with flow rate at 500 cc/min. so column No. 4 was inserted and influent became negative at once. Flow rate was maintained constant at 500 cc/min. for 76 hours, resulting in 8.6 complete changes of the menstruum. The effluent was still weakly positive at the end of 76 hours. No difficulty from the development of back pressure was encountered throughout.

Recovery: Alkali regeneration was performed according to standard method outlined for EB2-8 series, as was acid regeneration.

Alcohol stripping differed from that employed in EB2-8A in omitting the 3 liter wash with hot alcohol.

Before concentration the alcohol elutriant was adjusted to a pH of 5 - 6.5, it having shown a value of 10-12 when drained from the column. 305.58 grams of solid material were recovered from the distillation, only 71.55 grams of which were from the combined first wash lots (12 liters).

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 8B

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 13 Feb 45 CONTINUED:

Recovery:

NOTE:

At the end of the run it was discovered that a considerable percentage of the fines had worked down through the bottom of the macerator. An assay of these fines showed an extraction of only 46 percent. This was undoubtedly due to the fact that the material had not been in proper contact with the fresh acid. The bark left in the macerator had been extracted 100 percent. A grain size analysis of the escaped material proved that 100 percent passed No. 70 mesh. Since the original material contained 86 percent of this size and the residual bark in the tank 59 percent, then with X equal to the weight of bark remaining in the macerator 10 - X equals the weight passing. In the original bark 14 percent, or 1.4 pounds, was retainable, since this amount represents 41 percent of what was actually retained and 14 percent of the original, therefore

$$.41\ X = (.14 \times 10)$$

$$X = \frac{.14 \times 10}{.41} = 3.4 \text{ lbs. of material}$$

retained on the macerator bottom. Thus 6.6 pounds escaped and was extracted only 46 percent, which amounts to 64.37 percent of the total available alkaloid content of the original.

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 8C

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 21 Feb 45

Pounds of Bark	10	Total Solids Recovered	305.3
Size of Grind	¼" Hammer mill	TA of Recvd Solids	66.6%
TA Original	5.56%	% TA Extd. & Recovered	96
TA Residual	*	% TA Orig. Recovered	86.5
% TA Extracted	90.0*		

*All fines escaping percolator recovered and assayed

OPERATIONS DATA			
Maceration Time	76 Hrs.	Conc. & Vol. of Menstruum	.1 N, 60 l.
Stirring Time	8 "	Conc. & Vol. Alkali Regen./Col.	.5 N, 12 l.
Circulation Time	20 "	Conc. & Vol. Acid. Regen./Col.	.5 N, 12 l.
No. Fluid Cycles	8.6	Rate of Application of Regen.	300 cc/min.
No. Columns	3	Elutriant, Vol./Col.	14 l.
Total Bed Volume	13.5 l.	Application of Elutriant	Cycled 2 Hr.

NOTES

Apparatus: Equipment assembled as in EB2-8A. A disk of vinyl coated cloth was placed in the bottom of the macerator during the stirring phase to prevent the loss of fines. This cloth was removed at the end of the stirring phase. At the end of the experiment it was found that about ten percent of the fines had worked through in spite of the precaution.

Extraction: Circulation was started through three ion exchange columns. Since the sample of menstruum at top of column No. 3 remained negative to Mayer's, the fourth unit was not added in this experiment. A flow rate of 500 cc/min. was maintained during the entire circulation phase. By the end of 76 hours the fluid taken from the macerator was faintly positive.

Recovery: Regeneration was accomplished by the standard method as outlined for EB2-8A, using .5 N NaOH.

Methyl alcohol was used as elutriant for this experiment instead of ethyl. It was found to be difficult with the apparatus used to bring the proof back to a level higher than 140 degrees. All alcohol was recovered in the same container, however 18.19 grams of pinkish white crystaline material was precipitated and recovered by decanting. This material proved to be 100% alkaloid. The combined alcohol lots distilled down from 48 l. (14 l. alcohol plus water per column) to four liters was evaporated to dryness and produced a total of 305.3 grams of total solids with a TA of 66.6%.

CINCHONA EXTRACTION - ION EXCHANGE, DATA SHEET

EXPT. NO. EB2- 8D

ENGINEER BOARD, FORT BELVOIR, VIRGINIA

Date 1 Mar 45

Pounds of Bark	10	Total Solids Recovered	266
Size of Grind	¼" Hammer mill.	TA of Recvd Solids	63.1%
TA Original	3.86%	% TA Extd. & Recovered	100 +
TA Residual	0.26%	% TA Orig. Recovered	100
% TA Extracted	93.00		

OPERATIONS DATA			
Maceration Time	100 Hr.	Conc. & Vol. of Menstruum	.1 N, 60 l.
Stirring Time	42 3/4"	Conc. & Vol. Alkali Regen./Col.	.5 N, 12 l.
Circulation Time	45 1/2"	Conc. & Vol. Acid. Regen./Col.	.5 N, 12 l.
No. Fluid Cycles	12.7	Rate of Application of Regen.	300 cc/min.
No. Columns	3	Elutriant, Vol./Col.	14 l.
Total Bed Volume	13.5 l.	Application of Elutriant	Cycled 2 Hr.

NOTES

Apparatus: In this experiment the monel filter shell used in EB2-8 was again brought into use and assembled in the same manner.

Extraction Deviations from the standard method as outlined in EB2-8A were introduced as follows. Circulation continued until the effluent was negative or nearly so. At the end of each circulation phase stirring was resumed and continued until one hour before circulation was to be resumed. The flow rate was limited by the mechanics of the apparatus, but attempts were made to strike the maximum short of break-through. The average was 780 cc/min.

Recovery: Regeneration was performed by the standard method. Methyl alcohol was again used instead of ethyl.

266.0 grams of solids were recovered from concentration of the alcohol. This material had a TA of 63.1 percent. No separation of the concentrates was attempted.

ANALYTICAL METHODS

Throughout the course of investigational work on cinchona extraction it was necessary, in order to evaluate results, to perform systematic determinations of the percentage of alkaloids contained in the original bark, the extracted bark, and the end products or totaquines.

In commercial laboratory practice it is customary to perform determinations of the separate alkaloids, quinine, cinchonidine, cinchonine and quinidine. However, in the elaboration of an extraction process the separation of the four alkaloids is of no importance since their proportion in either the extract or the residual amount remaining in the treated material will depend entirely on the kind and quality of the original bark. Repeated assays of treated bark in which such separations were made seem to prove that preferential extraction does not occur. Since separation of the four principal alkaloids is a time-consuming task requiring special equipment, it was decided that, in view of the existence of a standard method of total alkaloid determination, the separations would not be attempted. Accordingly, the Dutch Titration Method for the Determination of the Total Quantity of Alkaloids in Cinchona Bark was adopted and, with certain modifications, used exclusively for all assays made at the Engineer Board laboratories.

The following is an excerpt from the printed report of the Committee for Cinchona Analysis, date Amsterdam, 28th February 1920, as translated from the Dutch:

(p.9) "Method for Analysis of Cinchona Bark "

"I. The Setting free of the Alkaloids in the Bark

Wanted: 1. Slaked lime, to pass test; 200 mgms. boiled with 25 cc H_2O in which 4 gms. NH_4Cl have been dissolved. Should give practically clear solution. Lime freed of coarseness by sifting.

2. Soda lye (50 gms. NaOH/L) S.G. at 15° = 1.055 20 gms. bark powder B-30,-B-40 are well mixed with 6 gms. slaked lime in porcelain cup by means of spatula until an apparently homogeneous mixture is obtained.

"II. Extraction

Wanted: 1. Benzoline boiling 80-81° coagulating in ice. (Need not be throphine free)

2. Cotton wool (grease-free) Rough powder transferred quant. from cup to extra tube (f.i. one of Schlucher and Schiill #603) Draw 43 mm. length 123 mm., bottom of which has been previously provided with wad of cotton wool.

Cup cleaned thoroughly and wiped out with cotton moistened with benzoline.

Thimble put into extractor and ± 300 cc C_6H_6 added. Boiled 8 hours as vigorously as possible.

(p. 11) "III. To change the alkaloids into their HCl acid salts

Wanted: (1) 1/1 N HCl

(2) Mayer's reactive

Distill off C_6H_6 until only little remains (water bath, bulb up to neck)

10 cc. N-HCl added from burette, mixed with 30 cc H_2O and remaining C_6H_6 distilled from water bath. The HCl will in this way ext. from the resinous mass, the whole of the alkaloids. HCl solution filtered through water-moistened wad cotton into calibered cup of ± 150 cc. capacity.

Bulb and funnel repeat. rinsed with distilled H_2O (small quantity each time) until total volume equals ± 75 cc. Last drops may no longer react on Mayer's.

"IV. To determine the total quantity alkaloids

Wanted: (1) 1/1 N soda lye

(2) Litmus paper answer. require of the Netherland Pharmacopoeia, Ed. IV, vi 2, that a drop of a 1/1000 N alk. solution shall immediately turn it blue.

The liquid above (acid solution) is just put to boil on a small stove (hot plate, etc.) and excess HCl titrated with 1/1 N soda lye. End pt. fixed for stippling on litmus paper. While titrating, keep as hot as possible. Use thin, pointed stirring rod.

"X. Calculation of the Analyses

The total quantity of alkaloids is expressed by $\frac{(10-V)\ 3.1}{2}$ % when V is the number of cc. of 1/1 N soda lye. used for the reverse titration."

Modifications: It was found that with certain lots of the bark used in the Acid Extraction Experiments, the extraction of 20-gram samples for assaying produced so great a precipitation of extraneous materials during the process of titration as seriously to obscure the end point. After repeated attempts to obtain consistency within the allowable .25 percent margin of error, it was decided to reduce the size of the sample to 10 grams. An examination of the method of calculating analytical results indicates that the reduction in quantity of bark causes the error to increase. The formula for such computation is:

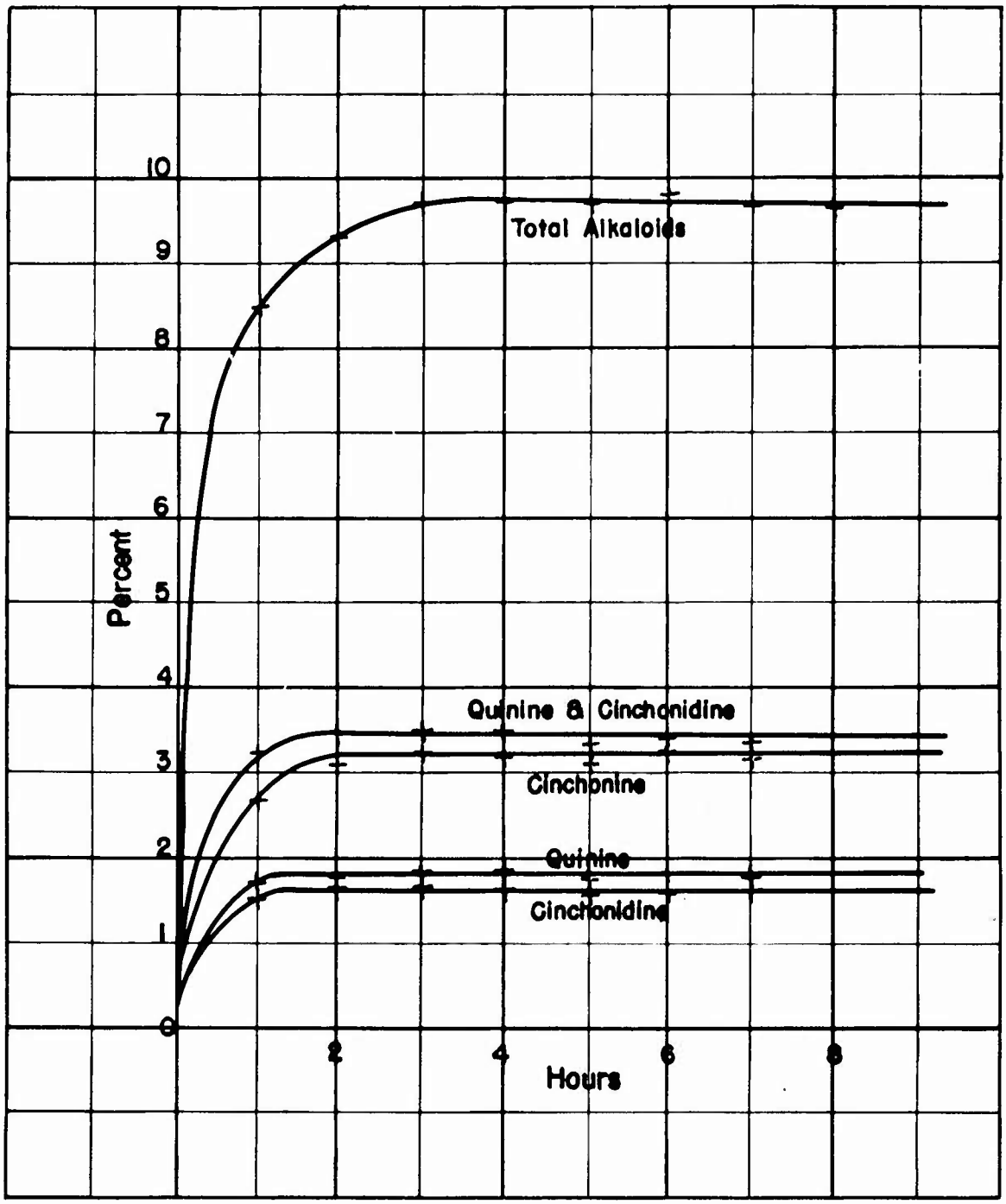
$$(\text{meq. HCl}) - (\text{meq. NaOH}) \times \frac{3.1}{2.0} = \text{percent TA}$$

in which 3.1 represents the average molecular weight of the alkaloids (310) and 2.0 represents the 20-gram sample so arranged as to place the result of the computation directly in terms of percentage. It is obvious that dividing the denominator of the formula by two is equivalent to multiplying the numerator by the same number. The error in reading the burette is increased by reducing the volume required for the titration and again by reducing the size of the sample since the burette readings also appear in the numerator.

The error in the assumed molecular weight does not affect the comparative values in duplicate assays since it is a constant. In the case of computations for percentage extraction or recovery, the molecular weight is of no significance since it appears on both sides of the equation and is cancelled out. In the evaluation of bark and concentrates, however, the error resulting from inaccuracy of the assumed molecular weight could be as high as 20 percent, though it is more likely to be in the neighborhood of 5-6 percent as in barks of low quinine content. The sources of error which directly influence possible variation between duplicate determinations are (1) the difficulty experienced in detecting satisfactory end point in the titration, and (2) the expected error in burette reading which is commonly considered to be $\pm$ .02 ml. for each reading. The error in the gravimetric determination is of no consequence here since it occurs in the fourth decimal place and hence does not enter the calculations.

Though the end point of the titration was clarified by the reduction in the weight of the bark sample, it proved still to be difficult to determine consistently. In view of this and considering the apparent increase in magnitude of the burette error, it was considered advisable to raise the allowable margin of error to .4 percent. It was found to be possible to observe this limit.

Further modification consisted of the substitution of hydrion paper as a more efficient indicator than the litmus paper



SOXHLET EXTRACTION-TIME CURVES

FIG. 25.

A 6076

specified by the Dutch and reduction of the minimum extracting time to four hours. It was found that a negative Mayer's test could be obtained from the extraction thimble in four hours in nearly all cases. A report on extraction time studies by Dr. Durand, Rutgers University College of Pharmacy is given below:

"Time Required to Extract Alkaloids
Professor E. M. Durand, Rutgers University"

"In this series of experiments, the results of which are shown on the accompanying graph, 20 grams of bark (30-40 mesh) were mixed well with 6 grams of slaked lime. Then 20 cc. of 5% sodium hydroxide were added and the whole again mixed well. The mixed bark was transferred to a thimble and extracted with benzene in a Soxhlet extractor.

"An average time for the period of one cycle was about two minutes. There were variations in time in the series of from $1\frac{1}{2}$ to $2\frac{1}{2}$ minutes for a cycle however.

"According to the figures so far obtained, it would seem that all of the alkaloids in any measurable amount are extracted in three hours. The percentages shown are percentages of alkaloids in the bark.

"At the end of two hours 98.3% of the T.C.A. present is extracted. This breaks down as follows: 97.3% of the quinine, 100% of the cinchonidine and 98.1% of the cinchonine.

"We have just started another series of experiments in which we mix 40 grams of white seashore sand with the bark after the alkali is thoroughly mixed. We put a 1 cm. layer of sand in the bottom of the thimble. So far we have only the data on total alkaloids extracted. This figure is 9.16% in one hour as against 8.49% in one hour without the use of sand.

"It is our feeling that channeling may occur and the benzene may not come into contact with the bark in the thimble. Our use of sand is designed to overcome this possible difficulty.

"In another experiment that we ran some time ago, we found that in the first five cycles 72.2% of the alkaloids came out; in the next five cycles, 17.4%; in the next five cycles 7.0%; in the next five cycles, 2.6%; and in the next five cycles 0.8%."

"These last figures were all on total alkaloids as determined by titrating with normal acid and alkali. Between the second five cycles and the third five, the extraction was shut off overnight. This gave the bark a chance to soak in the benzene and so possibly facilitated the complete extraction in the twenty-five cycles."

Assay of Concentrates: No standard method for total alkaloid determination of alkaloid concentrates was available, so an adaptation of the Dutch Titration Method was used.

Concentrates from the ion exchange recovery method have, in general, an alkaloid content above 50% and often range as high as 80%. Thus, one gram of concentrate may have an alkaloid content as great as is found in 20 grams of bark. Accordingly the size of the sample was reduced to one gram. To reduce the value further would have brought the gravimetric error into the calculations, which would have tended to offset the advant ge gained in further clarifying the endpoint. The reduction to a 1 gram sample increases the magnitude of the burette error tenfold, so an allowable deviation between duplicate samples of some 4% had to be observed.

The marked tendency of samples of concentrate to form an almost impervious lump in the bottom of the extraction thimble was reflected in deviations amounting to as high as 20%. Since the tendency to form in lumps seemed directly connected with the practice of mixing 5 percent NaOH with the sample, the first attempts toward eliminating this source of error consisted of reducing the amount of hydroxide used. This practice proved to be unfeasible since a considerable portion of the concentrate is in the form of the acid salts. Being extracted as such, the presence of the alkaloid was not reflected in the titration. On the contrary, it proved desirable to use a considerable excess of hydroxide in order to assure the conversion of the alkaloid to the form of the natural base. The problem of lumping was solved by mixing the concentrate sample with 10 grams of diatomaceous silica, 95% of which passes 150 mesh. This amount of silica produces a bulk approximately that of 10 grams of ground cinchona bark. Thorough mixing of the sample (70 mesh) with the silica provides a fine pervious mass in which the tendency toward channelling is minimized. Examination of the extracted material taken from the Soxhlet thimbles proved that it had lost none of its cohesionless character. The porousity of the material made possible the use of 10 cc of 5 percent NaOH, an amount sufficient to insure conversion of all salts present. Early attempts to spread the concentrate on layers of cotton, both before and after addition of the hydroxide, proved difficult and produced no consistent improvement in final results. The addition of slaked lime was continued as standard practice though, in view of its function in bark extraction, it probably accomplishes no purpose.

An alternate gravimetric method of total alkaloid determination used by the Dutch for the so-called pharmaceutical barks is shown below. Here a partial separation of the alkaloids is made, the determinations being performed on the mixed tartrates of quinine and cinchonidine plus the extracted cinchonine and a mixture of the quinidine and amorphous alkaloids.

The chief advantage entailed in this method is that for most South American barks a value may be derived in which the amorphous alkaloid does not appear. In the case of totaquines, this permits a direct evaluation in terms of U.S.P. specifications with the exception of the quinine content which will, in any case, depend on the quality of the bark extracted. The following excerpt is from the Report of Chemistry for Cinchona Analysis.

"VIII. To Determine the Total Quantity of Alkaloids in Pharmaceutical Barks.

Wanted: 1. Diluted HCl
2. Litmus paper
3. Na_2CO_3 (5%)
4. $CHCl_3$, answer. require Netherland Pharmacopoeia
5. Ammonia (10%)
6. Spirit (96%)
7. Mayer's reactive

The cinchonine crystals obtained under VII,

VII. To determine quantity of cinchonidine in barks which are rich in cinchonine (succirubra and robusta). Wanted: (1) Alcohol 96%, (2) Ammonia 10%.

'If barks are rich in cinchonine, this fact often betrays itself by small crystals detaching themselves in the course of extraction in the boiling C_6H_6. The cinchonine must then be removed to a large extent by the following process.

' The HCl Alkaloids solution obtained under III is reduced by evaporation to 50 cc. in small Ostwald Stove; Mixt. 45 cc. alc. and 5 cc. ammonia is added. The cinchonine then appears in crystallized form; if necessary a small crystal of cinchonine may be used for inoculation.

' After 24 hours the liquid is filtered through a funnel with a small wad of cotton wool and subsequently, cup and funnel repeatedly rinsed with a total of 25 cc. of a mixture of equal vol. parts of a 96% alcohol and water, to which one or two drops of ammonia have been added.'

which have accumulated wholly or partly on the wad of cotton wool, are dissolved in 5 cc. diluted HCl solution, by pouring

HCl over them and catching the solution and moderately used wash water in a cup in which the cinchonine had been separated. After filtration of the tartrates the mother lyc. of the tartrates is subsequently put quantit. in the same cup and the liquid neutralized on the H_2O bath (litmus paper as indicator) with the Na_2CO_3 solution. After cooling, the solution is filtered off in separatory funnel, and 100 cc. $CHCl_3$ are added with 10 cc. ammonia. The whole is then shaken until the watery liquid on top 1 cc. acidulated with HCl no longer shows any reaction with Mayer's. Should it appear, even after repeated shaking, that this reaction remains, a further 100 cc. of $CHCl_3$ are filtered into funnel and shaking renewed until no sediment appears with Mayer's.

Of the $CHCl_3$ 80 (resp. 160 cc.) are taken, transferred to a weighed flask, $CHCl_3$ distilled off and residue left in flask covered with 5 cc. spirit (96%) this is evaporated on H_2O bath, after which flask and contents are dried at 110° C, and after cooling in desiccator, weighed. See further under X.

"X. Calculation of the Analyses

If the total quantity of alkaloids is determined, in conformity with the description under VIII, then the increase in weight of bulb, (flask) multiplied by 1.25 will indicate the quantity of cinchonine and amorphous alkaloids in 20 grams bark. The total contents of alkaloids (in %'s) is ascertained by multiplying this quantity by 5 and adding same to ascertained % contents of quinine and cinchonidine."

TO: ROBERT LEE KAYE, MAJOR, CE

FROM: ERNEST H. SIEVEKA - ENGINEER, CHEMICAL

PROJECT: WSS 530, ENGINEER BOARD, FORT BELVOIR, VA.

SUBJECT: THE DETERMINATION OF NITROGEN IN TOTAQUINES

THE DETERMINATION OF NITROGEN IN TOTAQUINES

by Ernest H. Sieveka

The four commercially important cinchona alkaloids have very similar molecular structure, being ditertiary amines composed of a quinoline nucleus with a complex sidechain containing one of the two nitrogen atoms of the molecule. It follows, therefore, that the nitrogen concentration, if accurately determined, provides a measure of the amount of alkaloid present in a sample, within the margin of error imposed by the variance of molecular weights. A study of the methods for determining nitrogen was made with the view of finding a satisfactory analytical procedure. As in all analytical work, a method was desired by which determinations could be made in a minimum amount of time with the least amount of manipulation. Direct Nesslerization following Kjeldahl digestion provides a rapid method for measuring nitrogen as ammonia and was given first consideration. Readings of ammonia concentration in the Nesslerized solution were made on a Fisher Electrophotometer using a 425 millimicron filter. The procedure for Nesslerization and calibration of the electrophotometer were taken from "Colorimetric Analysis with AC Model Fisher Electrophotometer", Fisher Scientific Co., as given under "Method for the Determination of Non-Protein Nitrogen in Blood". This procedure is based on the work of Folin and Wu, J., Biol. Chem. 33, 81 (1919) and Northrup, J. Lab. & Clin. Med. 24, 433 (1939). Those portions of the method pertinent to the present determinations are submitted as follows:

"NON PROTEIN NITROGEN IN BLOOD "

"Solutions Required:

NESSLER'S STOCK SOLUTION. SOLUTION NO. FE-32. Place 75 g of potassium iodide, 55 g of resublimed iodine, 50 ml of distilled water, and about 75 g of redistilled mercury in a 500 ml Florence flask. Shake vigorously until the iodine is nearly dissolved. When the solution has begun to pale, cool in running water and continue shaking until the reddish color is replaced by the greenish color of the double iodide. Decant the liquid from the surplus mercury into a 1000 ml volumetric flask and wash the mercury several times with distilled water, adding the washings to the solution. Dilute to one liter. Store in a screw cap amber bottle.

NESSLER'S SOLUTION, DILUTE. SOLUTION NO. FE-25. To one part of the stock Nessler's solution (FE-32) and one part of water add four parts of 10% sodium hydroxide (FE-34). Store in amber, screw cap bottle.

SELENIUM DIGESTION MIXTURE. SOLUTION NO. FE-26. Add 100 mg of selenious acid to 200 ml of a mixture of 3 parts of concentrated sulfuric acid and 1 part phosphoric acid. Store in g.s. bottle.

NITROGEN STANDARD SOLUTION. SOLUTION NO. FE-27 5 ml = 1 mg N. Dry ammonium sulfate, C. P., at 100° C. Prepare a solution containing 0.0944 g per 100 ml. Store in screw cap bottle.

"Calibration:

Transfer 10 ml of the standard nitrogen solution to a 100 ml volumetric flask, dilute to the 100 ml mark with distilled water. This results in a dilute standard containing 0.02 mg per ml.

1. Transfer X ml of the diluted nitrogen standard to a Folin digestion tube. Dilute to 35 cc mark, using distilled water, and mix.

2. Bring volume to 50 ml mark with dilute Nessler's reagent and mix thoroughly.

3. Transfer to a Cylindrical Absorption Cell and determine the Electrophotometer scale reading, after the Initial Null adjustment has been made with the reference cell containing a blank consisting of a mixture of 17.5 parts of distilled water to 7.5 parts of Nessler's reagent.

The following quantities of standard are suggested as values of X for the preparation of the calibration curve:

Ml. diluted N.P.N. standard (0.02 mg per ml)	Ml water	Equivalent mg N.P.N. per 100 ml blood
1.0	34	6.7
2.0	33	13.3
3.0	32	20.0
4.0	31	26.7
5.0	30	33.3

Plot the results on graph paper. Calculate the factor or prepare a calibration curve.

"Analysis:

1. Transfer 3 ml of protein free blood filtrate, prepared as described in instructions on determination of Sugar in Blood, to a Pyrex glass tube graduated at 35 and 50 ml.

2. Add 1 ml of selenium digestion mixture.

3. Heat gently with a micro burner until the tube is filled with white fumes.

4. Reduce the flame and continue heating until the solution is clear.

5. Cool and dilute to 35 ml.

6. Bring final volume to 50 ml mark with dilute Nessler's solution.

7. Mix well and transfer about 25 ml of the solution to a Cylindrical Absorption Cell.

8. Determine the scale reading after the Initial Null adjustment has been made, the reference cell containing a mixture of 17.5 parts of distilled water and 7.5 parts of Nessler's reagent.

9. From the observed scale reading, determine the N.P.N. concentration by the use of the appropriate factor or reference to the chart."

Selenium dioxide was substituted for the selenious acid given in the Fisher procedure. In addition to the selenium digestion mixture, a copper selenate digestion mixture was also prepared to be used in parallel with the selenium dioxide mixture. The only essential difference is the presence of copper sulfate in the latter. The selenium dioxide used in both digestion mixtures and the copper selenate solution were prepared according to Hinman, Weber and Kountz, "Ammonia and Organic Nitrogen Determinations in Stream Pollution Studies", Iowa Academy of Science, 1940. The following is taken from the above article:

"COPPER SELENATE CATALYST SOLUTION "

"Powdered black elementary selenium is oxidized by the aid of nitric acid and heat. About 25 grams of the substance is placed in a one-liter flask under a hood and dilute nitric acid (1:1) is slowly added until action ceases. Heat is then cautiously applied. It is important to use a large flask because the mixture foams vigorously. The poisonous nature of selenium and of selenium fumes should be kept in mind. Excess oxides of nitrogen must be removed from the material by evaporating the mixture to dryness, dissolving in water and repeating the evaporation to dryness at least three times. The residue must be white. Any yellow color indicates failure to remove all of the nitrogen oxides resulting from oxidation of the selenium. This calls for another dissolving of the residue and another evaporation to dryness.

"One gram of the dried selenium oxide, prepared as above, and one gram of copper sulfate of analytical grade, are to be dissolved in 100 milliliters of a mixture of one part syrupy phosphoric acid and three parts of concentrated sulfuric acid, nitrogen free. Mr. Berry's procedure, which expedites the preparation of the reagent, consists of dissolving the selenium oxide and the copper sulfate separately in the minimum quantity of ammonia-free distilled water and then adding, first, the selenium solution, and second, the copper solution to the required quantity of phosphoric acid, (25 milliliters for each 100 milliliters to be prepared). Finally the concentrated sulfuric acid is added to bring the solution to the predetermined volume.

"The resulting copper selenate catalyst solution is very poisonous as well as very corrosive."

The two digestion mixtures were used in parallel during the Nesslerization studies to determine if the copper selenate might be a more effective catalyst than selenium dioxide.

Trial determinations using the Nesslerization method were made on standard samples of quinidine sulfate. It was obvious that direct Nesslerization as carried out in the Fisher procedure was not adapted to a solution in which a copper catalyst had been used since the precipitate of $Cu(OH)_2$ formed on Nesslerization would produce an error in the electrophotometer readings. Any turbidity present in the digested solution whether originating from the presence of copper or other foreign substances produces an error in the photometer reading.

In view of the turbidity interferences in making readings, the direct Nesslerization method for ammonia as applied in water analysis was adapted to the Fisher procedure. Details of the procedure for the direct Nesslerization of ammonia in water is found in "Standard Methods of Water Analysis" 8th Ed. 1936, pages 128-129.

The procedure used for the determination of nitrogen in quinine sulfate is outlined as follows:

a. Approximately one gram of quinidine sulfate was made up to a liter with ammonia-free water. The solution was acidified with H_2SO_4.

b. A 50 ml aliquot was treated with 10 ml of selenium dioxide digestion mixture or 10 ml of copper selenate digestion mixture and digested for approximately 15 minutes after a clear solution is obtained, in accordance with the procedure given by the Fisher instruction manual.

c. The digested solution was transferred to a 250 ml or 500 ml volumetric flask and made up to volume with ammonia-free water.

d. For direct Nesslerization 10 ml, 20 ml, or 25 ml portions, depending on the concentration of the aliquot, were made up to 35 ml with ammonia-free water. Fifteen ml of Nessler's solution were added and readings taken immediately on the photometer. The Nessler's solution added to the sample was filtered to remove suspended matter.

e. In applying the direct Nesslerization method as used for ammonia in water, 50 ml or 100 ml of the digested solution (diluted to 250 ml or 500 ml) depending on the amount of nitrogen present are placed in a tall 100 ml Nessler tube. One ml of copper sulfate (100 g per liter) is added. Sodium hydroxide solution (50%) is added until the solution is just alkaline. Considerable excess will cause poor floc formation. After careful mixing to avoid loss of ammonia, the tube is stoppered and allowed to stand until the supernatant is entirely clear and free from turbidity. This may require 24 or 36 hours. An aliquot is then withdrawn from the supernatant and Nesslerization is then performed as in d above.

To check the effectiveness of the Nesslerization method and the efficiency of the Kjeldahl digestion, the ammonia obtained by similar digestion was measured by distillation and titration. 100 ml or 200 ml aliquots of standard quinidine sulfate were digested in the same manner as for direct Nesslerization. The digestion residue was diluted with ammonia-free water, made alkaline with NaOH, the ammonia distilled into 0.01 N H_2SO_4 and the excess acid back-titrated with 0.01 N sodium carbonate using methyl red as an indicator. Bromcresol green was later found to give a much more "readable" endpoint in the work.

Nitrogen values obtained by the above procedures on quinidine sulfate are given in Table I.

Preliminary work on pure quinidine sulfate using a very limited digestion period indicated that an average of 50% of the theoretical nitrogen in the alkaloid molecule is recovered. The early results made it apparent that one of the nitrogen atoms of the alkaloid molecule is readily converted to ammonia while the second, which is part of the quinoline structure within the molecule is considerably more resistant to oxidation as would be expected. The procedure followed for the nitrogen determinations in Table I is similar to the official Kjeldahl method (Assoc. Official Agr. Chem., Official and Technical Methods of Analysis, 5th Ed., p 26 (1940)) using selenium dioxide as a catalyst instead of mercuric oxide. Two very important exceptions to the official method should be noted. The time of digestion is short, less than half an hour, while the official method calls for as much as two hours of digestion. Likewise, the K_2SO_4 or Na_2SO_4 added to raise the temperature is omitted. Results obtained by such limited digestion covering quinidine sulfate are given in Table I. Totaquine values are given in Table II. These results indicate that the second nitrogen atom cannot be quantitatively recovered by mild digestion and hence rapid Kjeldahl digestion followed by rapid direct Nesslerization appears unsatisfactory as a method of analysis. The totaquine nitrogen values show that partial liberation of the second nitrogen atom takes place even on short digestion periods. Hence the assumption that 50% of the available nitrogen can be quantitatively recovered from totaquines by short Kjeldahl digestion is not warranted.

Literature references indicate that quinine liberates ammonia on acid permanganate oxidation. The extent to which nitrogen is liberated from the alkaloid molecule on mild oxidation was investigated by treating several Fort Belvoir totaquine samples according to the following procedure:

Acid Permanganate Oxidation -

One gram of totaquine was washed into the Kjeldahl flask with H_2SO_4 (approx 0.5 N). Ten ml concentrated H_2SO_4 and 10 g $KMnO_4$ were added. Emphasis was placed on adding the acid first. One hour digestion was given on a boiling water bath. The solution was cooled, made alkaline, and distilled into 0.1 N H_2SO_4.

Results obtained by titration of ammonia give nitrogen values (Table II) corresponding to roughly 60 percent of the total alkaloid present.

Nitrogen values obtained in the initial distillation and Nesslerization tests indicated that a Kjeldahl procedure was needed which would liberate all of the nitrogen from the alkaloid molecule in a reasonable length of time, preferably not exceeding the two hour digestion recommended in the standard methods. Nitrogen determinations were made on quinidine sulfate, on an Ecuador totaquine and a totaquine produced at Fort Belvoir during the quinine extraction studies and termed the "Fort Belvoir Composite". The official Kjeldahl method, modified by using copper selenate digestion mixture as a catalyst in place of mercuric oxide and copper sulfate was employed. The digestion time was held at two hours for all samples.

A Kjeldahl modification using the reducing action of potassium iodide and sulfuric acid prior to the final digestion with the copper selenate mixture was carried out on the same series of samples. The potassium iodide treatment was taken from "Determination of Nitrogen in Nitriles", E. L. Rose and H. Zeliotto, Chemical Warfare Service, Edgewood Arsenal, Ind. & Eng. Chem., Analytical Edition, Vol. 17, No. 4, April, 1945. The following is a copy of the method of analysis used by the above authors:

"METHOD OF ANALYSIS"

"Place a weighed quantity of sample containing 40 to 60 mg of nitrogen in a digestion flask, add 1.5 grams of potassium iodide and 30 ml of concentrated sulfuric acid, and heat on a steam bath for 45 minutes with occasional shaking. Add 10 grams of potassium sulfate, 0.3 gram of anhydrous copper sulfate, 0.1 gram of selenium, and several boiling stones or glass beads. Heat the mixture gently at first, then boil briskly, and continue digestion for one hour after mixture becomes clear green in color. At no time should the volume of acid drop below 20 ml. Most of the iodine will have been removed by this time, but any remaining in the neck can be removed by applying a flame thereto. Cool the mixture, add 250 ml of water, and when cool proceed with the distillation. Add sufficient 50 percent caustic solution to make the reaction strongly alkaline (90 ml are usually sufficient), pouring it down the side of the flask, so that it does not mix at once with the acid solution. Add several pellets of zinc (20-mesh) to prevent bumping and without delay connect the flask to the condenser by means of the Kjeldahl connecting bulb, taking care that the tip of the condenser extends below the surface of a measured quantity of standard 0.1 N sulfuric acid. Mix the contents of the flask and distill until all ammonia has passed over into the acid. Usually 125 to 150 ml of distillate will contain all the ammonia. Titrate the distillate with standard 0.1 N sodium hydroxide solution, using sodium alizarin sulfonate mixed indicator. The end point is indicated by a change from green to bluish-gray color. Run a blank in the same manner as that used for the sample.

$$\frac{(\text{Ml of } H_2SO_4 \times N - \text{ml of NaOH} \times N) \times 1.401}{\text{weight of sample}} = \% \text{ N}"$$

Another modification tried consisted of adding sucrose to the alkaloid sample at the beginning of the regular Kjeldahl digestion.

For the totaquine samples treated by the various Kjeldahl methods the following procedures were employed:

a. Ordinary Kjeldahl digestion: One gram of sample is placed in a Kjeldahl flask by washing all the contents of the weighing bottle into the flask with 100 ml of approximately 0.5 N H_2SO_4; 20 ml of concentrated H_2SO_4, 10 ml of copper selenate digestion mixture, and 10 g of Na_2SO_4 are added. The solution is digested for two hours after a clear green solution is obtained. The digestion solution is diluted with ammonia-free water, cooled, partially neutralized with 30 percent NaOH and cooled again, and made alkaline to phenolphthalein with a slight excess. The ammonia is distilled into slightly more than the theoretical amount of 0.1 N H_2SO_4 required to react with the NH_3 present. The excess acid is back-titrated with 0.1 N NaOH using bromcresol green as indicator.

b. Sucrose Modification: In the sucrose modification, 0.5 gram of sucrose was added directly to the digestion flask and the procedure carried out in exactly the same manner as for the ordinary Kjeldahl digestion. If the amount of sugar in proportion to the acid is not excessive, no difficulty will be encountered with frothing and foaming. Should this occur, the difficulty may be overcome by cooling and adding a small amount of water. Very slow heating is helpful if slight frothing occurs.

c. Potassium Iodide Modification: In the potassium iodide modification, 1.5 g of potassium iodide and 20 ml conc H_2SO_4 were added to the 1 g sample in the Kjeldahl flask containing approximately 100 ml of 0.5 N H_2SO_4. The sample was heated on a water bath for two hours. Following the water bath treatment, 10 ml copper selenate and 10 g potassium sulfate are added and the digestion is completed exactly as in the ordinary Kjeldahl procedure.

Table III covers the nitrogen values obtained on both totaquines by the three Kjeldahl methods.

A discussion of the procedures employed for the determination of nitrogen and an evaluation of the results obtained are summarized briefly as follows:

a. Kjeldahl Digestion. The results obtained by Kjeldahl digestion demonstrate that nitrogen can be quantitatively recovered from totaquines by this method. The time and temperature of digestion are important factors. The nitrogen values obtained by distillation on quinidine sulfate give an overall average deviation of approximately two percent from the theoretical alkaloid content. Data obtained on totaquines give nitrogen values corresponding to the total alkaloid obtained by the Dutch method indicating that the nitrogen is liberated from all of the alkaloids present in the totaquine including both the

amorphous and crystallizeable forms. Deviations from the values obtained by the Dutch Method vary on the average by two percent to four percent.[1] The average is slightly higher than the Dutch value.

b. Nesslerization. Nitrogen readings following direct Nesslerization are influenced considerably by the presence of turbidity. Nesslerized solutions, free from turbidity are obtained by a precipitation with $Cu(OH)_2$ prior to direct Nesslerization. Excellent nitrogen readings are obtained by the latter method but a long period of time is required for the precipitate to settle. Considerable manipulation and technique are required for carrying out the tests. Only a small amount of the total nitrogen (0.1 mg or less) in a sample can be measured which results in a large multiplication factor.

c. Distillation and Titration. Distillation and titration of the ammonia is considered superior to Nesslerization because the quantity of ammonia measured is larger (varying from 10 to 100 mg as compared with 0.1 mg by Nesslerization); the time consumed is less than that required by the copper hydroxide direct Nesslerization method, and the manipulation of the test is simpler.

Further Study. To establish the nitrogen determination as an assay method for totaquines, a number of comparative analyses with both the Dutch Method and nitrogen method are needed on a variety of totaquine samples. The Kjeldahl digestion deserves further study to determine the effect of varying digestion times with the view of establishing a maximum desirable digestion period. The influence of reducing substances such as potassium iodide and sucrose in shortening the digestion time should be ascertained.

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[1] See Appendix C, Analytical Methods

TABLE I
NITROGEN DETERMINATION ON QUINIDINE SULFATE
Limited Kjeldahl Digestion*
(Direct Nesslerization - Distillation and Titration)

Direct Nesslerization

Sample	Treatment	Nitrogen (Nessler) mg.	Nitrogen (Theor.) mg.	Nitrogen Recovered %
Quinidine Sulfate				
Q-1	Selenium Dioxide	0.044	0.072	61.0
Q-2	Catalyst	0.043	0.072	60.0
Q-1	Copper Selenate	0.058	0.072	80.5
Q-2	Catalyst	0.045	0.072	62.5
				Ave 71.0

Direct Nesslerization Preceded by Copper Sulfate and Sodium Hydroxide Treatment

Sample	Treatment	Nitrogen (Nessler) mg.	Nitrogen (Theor.) mg.	Nitrogen Recovered %
Quinidine Sulfate				
Q-1	Selenium Dioxide	0.036	0.072	50.0
	Catalyst	0.038	0.072	52.6
Q-2	Do	0.038	0.072	52.6
		0.036	0.072	50.0
Q-1	Copper Selenate	0.034	0.072	47.2
	Catalyst	0.034	0.072	47.2
Q-2	Do	0.038	0.072	52.6
		0.036	0.072	50.0
				Ave 50.2
Ammonium Sulfate				
N-1	Copper Selenate	.0124	.0124	100.0
N-2	Catalyst	.0129	.0124	103.0
N-3	Do	.1048	.1006	104.0
N-3		.1048	.1006	104.0
				Ave 103.0

Distillation and Titration

Sample	Treatment	Nitrogen Distilled mg.	Nitrogen (Theor.) mg.	Nitrogen Recovered %
Quinidine Sulfate				
1.0148 g/Liter	Copper Selenate	7.79	14.53	53.60
1.0021 g/Liter	Catalyst	6.55	14.35	45.59
1.0096 g/Liter	Do	7.20	14.45	49.79
1.0087 g/Liter	Do	7.31	14.44	50.54
				Ave 49.88
Ammonium Sulfate				
1.429 g/Liter	Digestion Omitted	2.937	3.02	97.20
1.473 g/Liter	Do	3.026	3.12	96.90
				Ave 97.05

Distillation and Titration
Quinidine Sulfate: - 200 ml aliquot digested and distilled
Ammonium Sulfate: - 10 ml aliquot made alkaline and distilled
Ammonia titrated with 0.01 N H_2SO_4 plus 0.01 N Na_2CO_3

Nesslerization
Standard Solutions
Q-1. 1.0021 g/Liter
Q-2. 1.0096 g/Liter
N-1. 0.1429 g/Liter
N-2. 0.1473 g/Liter
N-3. 0.9501 g/Liter

Procedure
Quinidine Sulfate - Aliquot digested - 25 ml. Digested aliquot diluted to 250 ml. Portion Nesslerized - 10 ml.
Ammonium Sulfate - Solution N-1 and N-2, aliquot digested - 10 ml. Digested aliquot diluted to 250 ml. Portion Nesslerized - 10 ml. Solution N-3 and N-4, 25 ml digested. Diluted to 250 ml. 5 ml Nesslerized.

*Kjeldahl Digestion -
Procedure according to "Colorimetric Analysis with A.C. Fisher Electrophotometer" Fisher Scientific Co. See text.
Copper selenate digestion mixture according to Hinman, Weeber and Kountz, Iowa Academy of Science, 1940.
Digestion Period limited to approximately 15 minutes after clear solution is obtained.

FIG. 26. NITROGEN DETERMINATION STUDIES

TABLE II
PRELIMINARY NITROGEN DETERMINATIONS
Quinidine Sulfate - Totaquine
(Kjeldahl Digestion - Permanganate Oxidation)

Quinidine Sulfate*
(Digestion time - 2 hours)

Sample Number	Treatment	Nitrogen Distilled mg	Nitrogen Recovered %
Q-1	Ordinary Kjeldahl Digestion	3.63	101.20
Q-2		3.59	100.07
Q-3		3.52	98.46
Q-4		3.48	96.90
Q-5		3.63	101.25
Q-6		3.55	98.88
			Ave 99.46
S-1	Sucrose Modification	3.53	98.50
S-2		3.60	100.20
S-3		3.53	98.50
S-4		3.70	103.20
S-5		3.53	97.93
			Ave 99.66
K-1	Potassium Iodide Modification	7.14	94.44
K-2		3.50	97.70
K-3		3.59	99.41
K-4		3.46	96.51
			Ave 98.26

Totaquine - Fort Belvoir Composite (T.A. = 90.36%)
(Limited digestion time - Approx. 15 minutes)

Sample Number	Weight of Sample	Portion Digested	Treatment	Nitrogen Distilled mg	Total Alkaloid %
1	2.0161 g/Liter	50 ml aliquot	Copper Selenate Catalyst (15 min digestion)	10.59	58.19
2	2.0041 g/Liter			10.66	58.90
3	2.0011 g/Liter			10.47	62.80
4	2.0024 g/Liter			10.69	64.75
5	1.0047 g.	Entire sample*		11.69	64.42
6	1.0167 g.			11.54	62.89
7	1.0127 g.			12.16	66.49
8	1.0074 g.			11.42	62.78

*Entire sample digested. Diluted to 500 ml. 50 ml aliquot distilled.

Totaquine - Fort Belvoir Composite
(Potassium Permanganate Oxidation)

1	1.0035 g.	--	$KMnO_4$ Acid Oxidation	52.22	57.61
2	1.0000 g.			54.60	60.46

* Standard Quinidine Sulfate Solution - 2.0061 g/liter. 50 ml aliquot digested and distilled.

(1) Ordinary Kjeldahl - Copper Selenate digestion mixture as catalyst. Digestion time - two hours.

(2) Sucrose Modification - 0.1 gm sucrose added at beginning of (1) ordinary Kjeldahl digestion.

(3) Potassium Iodide Modification - Predigestion on water bath for two hours with 1.5 gm KI followed by (1) ordinary Kjeldahl digestion.

(4) Potassium Permanganate - Gram sample acidified with H_2SO_4, ten gm $KMnO_4$, one hour digestion on water bath prior to distillation.

NOTE: Calculations on totaquines based on average molecular weight of alkaloid of 310. Titrations with 0.01 N H_2SO_4 and 0.01 N Na_2CO_3.

FIG. 27. NITROGEN DETERMINATION STUDIES

TABLE III
NITROGEN DETERMINATION IN TOTAQUINES
(Ordinary and Modified Kjeldahl Digestion - Distillation and Titration of Ammonia)

Totaquine - Fort Belvoir Composite (90.36% TA - Dutch Method)

Sample Number	Weight of Sample	Treatment	Nitrogen Distilled mg	Alkaloid by Nitrogen Method %
O-1	1.0022	Ordinary Kjeldahl Digestion	80.40	86.26
O-2	1.0042		78.40	86.45
O-3	1.0013		80.08	88.60
O-4	1.0029		81.48	89.97
O-5	1.0058		85.54	94.18
O-6	1.0012		84.98	94.00
O-7	0.9922		84.00	93.75
O-8	1.0207		86.14	93.45
O-9	1.0054		82.60	90.85
O-10	1.0036		84.40	93.15
O-11	1.0032		84.70	92.11
O-12	1.0082		86.24	94.76
O-13	1.0000		83.86	92.86
O-14	1.0018		80.92	89.45
				Ave 91.43
S-1	1.0014	Sucrose Modification	81.34	90.00
S-2	1.0074		82.32	90.50
S-3	1.0113		85.26	93.38
S-4	1.0125		86.52	94.64
				Ave 92.13
K-1	1.0043	Potassium Iodide Modification	83.58	92.17
K-2	1.0000		83.16	92.09
K-3	1.0012		88.76	98.18
K-4			88.34	97.50
				Ave 94.98

Totaquine - Ecuador Sample (66.46% TA - Dutch Method)

Sample Number	Weight of Sample	Treatment	Nitrogen Distilled mg	Alkaloid by Nitrogen Method %
O-1	1.0015	Ordinary Kjeldahl Digestion	62.44	69.07
O-2	1.0001		61.74	68.37
O-3	1.0013		62.00	68.58
O-4	1.0041		63.00	69.48
				Ave 68.87
S-1	1.0023	Sucrose Modification	64.26	71.00
S-2	1.0042		63.56	70.09
S-3	1.0135		63.14	69.00
S-4	1.0161		64.12	69.88
				Ave 69.99
K-1	1.0237	Potassium Iodide Modification	66.90	72.36
K-2	1.0524		68.04	71.60
K-3	1.0023		65.38	72.24
K-4	1.0018		67.62	74.07
				Ave 72.57

(1) Ordinary Kjeldahl - Copper Selenate digestion mixture as catalyst. Digestion time 2 hours.

(2) Sucrose Modification - 0.5 grams sucrose added at beginning of (1) ordinary Kjeldahl digestion.

(3) Potassium Iodide Modification - Predigestion on water bath for 2 hours with 1.5 g KI followed by (1) ordinary Kjeldahl digestion.

NOTE: All calculations based on average molecular weight of alkaloid of 310. Titration with 0.1 N H_2SO_4 and 0.1 NaOH.

FIG. 28. NITROGEN DETERMINATION STUDIES

TO: ROBERT LEE KAYE, MAJOR, C.E.

FROM: NORMAN APPLEZWEIG - CONSULTANT

PROJECT: WSS 530, ENGINEER BOARD, FORT BELVOIR, VA.

SUBJECT: ION EXCHANGE

1. INTRODUCTION

Following the loss of the Far Eastern sources of quinine and its raw materials, critical attention was focused on the utilization of South American and other available sources of cinchona bark. Unfortunately, the material available in this hemisphere is for the most part of much lower quality and alkaloid content than the East Indian bark. Consequently, the cost of extracting such low grade bark becomes a factor of prime importance.

The leaching of cinchona by dilute acid is a simple and inexpensive method of extraction which can be applied to low grade bark. However, recovery of the alkaloids from the aqueous menstruum, by alkali precipitation is time consuming and requires relatively large quantities of acid and alkali which are not recoverable.

As a possible means for improving the recovery of alkaloids, from acid solution, the use of adsorption was investigated.

2. ADSORPTION

The use of adsorbents for the removal and recovery of valuable ingredients from dilute solutions is a time-honored technique. It has the advantage that it permits the handling of large volumes of material in an economical fashion and often is the only way in which concentration of easily destroyed substances can be accomplished.

The ideal adsorbent for such a purpose is one which preferentially adsorbs the sought-after ingredient (together with a minimum of contaminants) and then permits its easy recovery in a concentrated and relatively pure state. The traditional adsorbents which have been employed are substances such as the colloidal clays, activated carbon, etc., which present a large surface area and possess chemically active groups which react with and bind the adsorbate.

Countless adsorbents have been used to remove alkaloids from solutions in organic solvents and several have lent themselves to use in aqueous media. Of the latter, activated carbon and the activated clays have been most successful.

In the case of carbon and clays, optimum adsorption requires carefully controlled conditions of pH so that the alkaloid is in its most adsorbable form, namely, under conditions of its least solubility in the fluid medium. Desorption can be accomplished by reversing these conditions and leaching the alkaloid from the adsorbent under conditions which make for its greatest solubility. Here, the adjustment of pH or the use of a better solvent are indicated.

While activated carbon will readily remove alkaloids from aqueous solutions under almost any conditions, the elutriation of the adsorbate cannot be accomplished efficiently enough to warrant its use in alkaloid recovery.

Lloyds' reagent has been used to remove alkaloids from neutral or acid solutions. The alkaloids were then extracted from the precipitate by means of an alkali and an organic solvent. (18)

Fink has suggested the use of a mixture of kaolin and asbestos as an adsorbent for cinchonine and quinine from dilute aqueous solutions. (6)

Ungerer reported the use of Calcium Permutit (probably $CaO.Al_2O_3.2\ SiO_2.6H_2O$) to take up quinine. (17)

3. BASE-EXCHANGE

The adsorption of alkaloids by all of the above-mentioned clays is undoubtedly a form of base-exchange. The sodium or calcium cations in the relatively insoluble clay are exchanged for the alkaloids, which act as cations when in the form of salts in aqueous solution. The alkaloid thus becomes part of the insoluble clay and is removed from the solution in contact with the adsorbent.

Nachod and Wood (14) describe a base exchanger as an anionic sponge filled with cations, the exchange of ions taking place in a mechanism similar to the game "musical chairs".

According to Myers (13) the ion exchange reaction, while it has customarily been referred to as an adsorption, actually must be primarily a chemical reaction. But this is so often marked by diffusion and reaction velocity factors that the practical equilibrium values obtained simulate adsorption values. In fact, ion exchangers exhibit typical Freundlich adsorption isotherms.

The base exchange phenomenon was first observed by Way in 1850 (19) who studied the adsorption of cations by soils. Eichorn in 1858 (4) observed that bases in cation exchangers were mutually exchangeable. Lemburg (11), Eichorn (4), and Gans (8) studied mineral exchanges extensively, and later Gans, made commercial applications of base exchange clays (Zeolites) to water conditioning.

Humus materials were found to be of value as cation exchangers by Fischer and Fuchs, who suggested that water could be softened by the sodium humates of brown coal (7).

While the Zeolitic clays capable of base exchange have enjoyed widespread application in commercial water softening for many years, the use of these adsorbents for other purposes has been limited by their

instability in acid media. The introduction of sulfonated coals (2) and specially treated resins (1) which were capable of cation exchange under any conditions of pH, and which would effectively exchange with hydrogen, opened vast new fields for the application of ion exchange.

With the consequent development of acid binding resin (1) or "anion exchangers" it now became possible completely to demineralize water (by exchanging H^+ from an acid treated cation exchanger for the cations in the water and then by adsorbing the acid thus formed on the anion exchanger).

Smit (15) has patented the use of ion-exchange adsorbents for the removal of electrolytes from solution of non-electrolytes, such as pharmaceutical preparations, dyestuffs and sugars. Gelatin solutions may be demineralized according to patent by Holmes (9) through passage over ion exchangers. Demineralization has also been suggested for the removal of salts from enzymes, glycerol solutions, and other solutions or organic materials (16).

Englis and Fiess who used demineralization in the production of a palatable artichoke syrup (5) noticed an appreciable diminution in nitrogen content as a result of this process. They then investigated the adsorption of amino acids by ion exchangers, and suggested that this was a possible means for their commercial recovery.

4. ION EXCHANGE IN ALKALOID RECOVERY

The newer ion exchange materials which are capable of the broad applications outlined above are also of especial interest in alkaloid recovery. Thus, while the zeolitic clays can be used only at conditions of pH suited to their stability, the hydrogen exchange materials can be used in strongly acid media and under rugged conditions.

An efficient method for recovery of alkaloids from dilute acid solution would permit the use of an economical acid-aqueous menstruum for leaching the alkaloids from the drugs containing them.

The use of a hydrogen exchange material ("ZeoKarb," a sulfonated coal) to recover quinine from acid solution, the breakthrough capacity of the exchanger for this alkaloid, and the use of ion exchange to concentrate the alkaloids in a totaquine preparation was reported by Applezweig (3).

The elutriation of the alkaloids from the exchanger was accomplished by means of alkali regeneration and an organic solvent in a manner similar to that of Waldbott (18).

The chart below illustrates the reactions involved:

HYDROGEN CYCLE

Adsorption	$HEx + MX \rightleftharpoons MEx + HX$	
Regeneration	$MEx + HX \rightleftharpoons HEx + MX$	

ALKALOID CYCLE

Adsorption	$HEx + Alk.\ H'X \rightleftharpoons Alk.H'Ex + HX$	
Regeneration	$Alk.H'Ex + MOH \rightleftharpoons \underline{Alk.} + MEx + H'X$	

Where alk.= alkaloid, Ex. = exchange adsorbent, M = cation)

The application of this technique to cinchona extraction by a cyclic system was suggested. Such a system would permit the acid percolate to pass through a column of exchanger and return to the percolator in a continuous fashion. In this way the drug would be constantly exposed to an extraction fluid free from alkaloids but saturated with respect to non-cationic ingredients.

The removal of a solid substance from solution by an adsorbent or exchanger can best be described by the Freundlich Isotherm $x/m = 1/kcn$ where "x/m" is the amount adsorbed per unit weight of adsorbent and "c", the residual concentration, "k" and "n" being constants characterizing the adsorbate as well as the adsorbent. Thus, it can be seen that substantial quantities of desirable materials are left behind in solution if a batch process is employed.

If, however, the continuous extraction process is used, as suggested above, fresh solvent is brought into contact with the bark, and the alkaloids in the resulting solution are completely removed by virtue of the quantitative action of the exchange material.

The successful development of this process at the Engineer Board has justified these predictions.

5. EXPERIMENTAL

The apparatus used consisted of a 25-gallon maceration tank, containing a percolator bag of canvas with a perforated metal bottom. The exchange columns consisted of 8.0 liter capacity stainless steel cylinders which were filled to a height of 8.5 inches with "ZeoKarb". The exchange columns were first converted to the acid condition by passing through the exchange bed 0.5 N sulfuric acid at a flow rate of approximately 300 ml. per minute. Previous to the starting of each new cycle this operation was repeated.

The bark was macerated with 0.1 N sulfuric acid, which was then cycled by being drawn off at the top, circulated "upflow" through the exchanger beds and back into the bottom of the maceration tanks by means of a pump.

Circulation was started with three columns in the line, the first being removed when saturated and a fourth added when necessary.

When the cycles were completed each column was regenerated with 12 liters of 0.5 normal sodium hydroxide and stripped of its alkaloid by displacing the water with 3 liters of alcohol, cycling 8 liters of alcohol for two hours and finally displacing with 3 liters of alcohol.

TABLE I

	Total Mac. Time Hrs.	Total Circ. Time Hrs.	TA Extracted %*	Efficiency of Recovery %**	Overall Recovery %***
1	76	20	---	---	63
2	76	20	64	100	76
3	76	20	90	96	86
4	100	20	93	100	100
Average	82 hrs.	20 hrs.	82.3%	98.6%	81.2%

* Computed on the basis of total alkaloid content of spent bark.
** Percentage of extracted alkaloids recovered.
*** Computed on the basis of total alkaloid content of orginal bark.

The alcohol was concentrated to dryness and the residue weighed and assayed for total alkaloid content.

In preliminary experiments it was found that continuous circulation had no advantage over an intermittent system which allowed more time for leaching of the alkaloids from the bark.

Periods of circulation were then alternated with periods of soaking, giving total maceration and circulation times as indicated in Table I. It was necessary to perform these experiments using dried bark. The preliminary soaking and stirring was employed in an attempt to prepare a simulated fresh bark. Field experience with acid extraction has indicated that the leaching of alkaloids from fresh bark is considerably more efficient than from dried (10).

6. DISCUSSION

That acid extraction is an economical method for cinchona alkaloid production has been amply demonstrated by Maranon and coworkers (12). The adaptation of acid extraction to field production units has

further extended the usefulness and economy of this method (10). The field production system permits the utilization of fresh bark and avoids the losses due to drying and shipping. Furthermore, it permits the production of inexpensive antimalarials for local use by a simple method using easily obtainable reagents and easily transportable equipment.

The application of ion exchange to the acid extraction system brings its overall efficiency and economy to a point where it can compete with or even surpass any of the methods now in use. The process requires the use of sulfuric acid and soda ash, both of which are easily obtainable in almost any part of the world. Ethyl alcohol, which is also used, is recoverable. The quantities of chemicals and the bulk of equipment required are considerably lower than that of the original acid extraction - alkali precipitation system (10).

Thus a process has been devised which will lend itself to large-scale permanent installation or small-scale, field production.

The economy, simplicity and efficiency of the Ion Exchange Acid Extraction System would seem to open many applications for this process. Its use for the production of inexpensive antimalarials for local use in Latin American and the Philippines seems indicated.

Since field production will permit the utilization of formerly inaccessible wild stands of cinchona, it would be possible to continue profitable exploitation of Latin American sources of cinchona alkaloids despite post-war competition from cinchona planters in the Dutch East Indies. Localities in which quinidine-rich bark is to be found may serve as a constant source for this vitally needed drug.

Another possibility would be to study the advisability of cultivating cinchona as a small two year old plant which can be sown and reaped by mechanical methods. This plan was tried by the Russians but overall yield was poor, since the green plants had to be dried before the alkaloids could be extracted by solvents. Acid extraction would permit the utilization of the entire fresh plant.

An inexpensive and continuous source of cinchona alkaloids would allow the development of an appreciable market for other than antimalarial applications.

Finally, there are many other alkaloid-bearing drugs of vital importance to the health and welfare of the Western Hemisphere which are native to this part of the world. The plants which yield atropine, scopolamine, strychnine, emetine, morphine, ephedrine, caffeine, nicotine, cocaine, colchicine and many drugs, all of which are found wild or can be cultivated in the Western Hemisphere.

SUMMARY & CONCLUSION

1. A cyclic system for the extraction of alkaloids from crude drugs has been devised.

2. The alkaloids of cinchona which were used in this demonstration were extracted by dilute acid and recovered by ion exchange with an overall average yield of 81.2% within an average period of 82 hours. Dried bark samples were used in these experiments. Greater efficiency may be anticipated when fresh bark is utilized.

3. Ion exchange fuctioned as a recovery system with an average efficiency of 98.6%.

4. The economy and simplicity of this method of extraction would seem to forecast its wide application to industrial alkaloid recovery.

5. The process as devised lends itself to small scale operation with simple, portable equipment and easily obtainable chemicals. Its application to field extraction would facilitate the utilization of formerly inaccessable wild cinchona stands and would eliminate substantial drying and transportation losses and costs.

6. The process should be of immediate value to the Philippine Islands and the Latin American Republics for the production of inexpensive antimalarials for local use.

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APPENDIX D

EQUIPMENT DESIGN

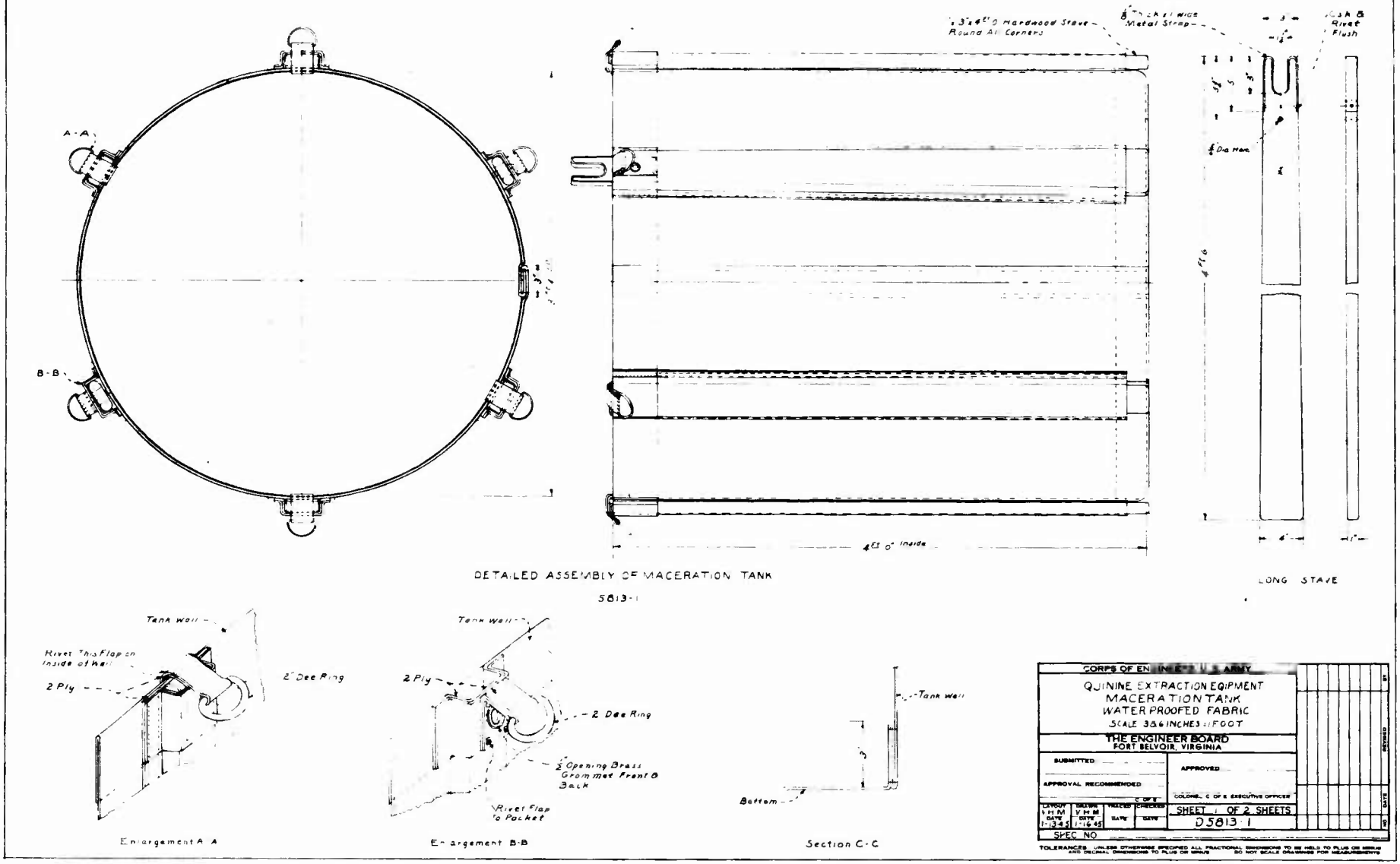


FIG. 29. PLAN, ELEVATION, DETAIL, MACERATION TANK

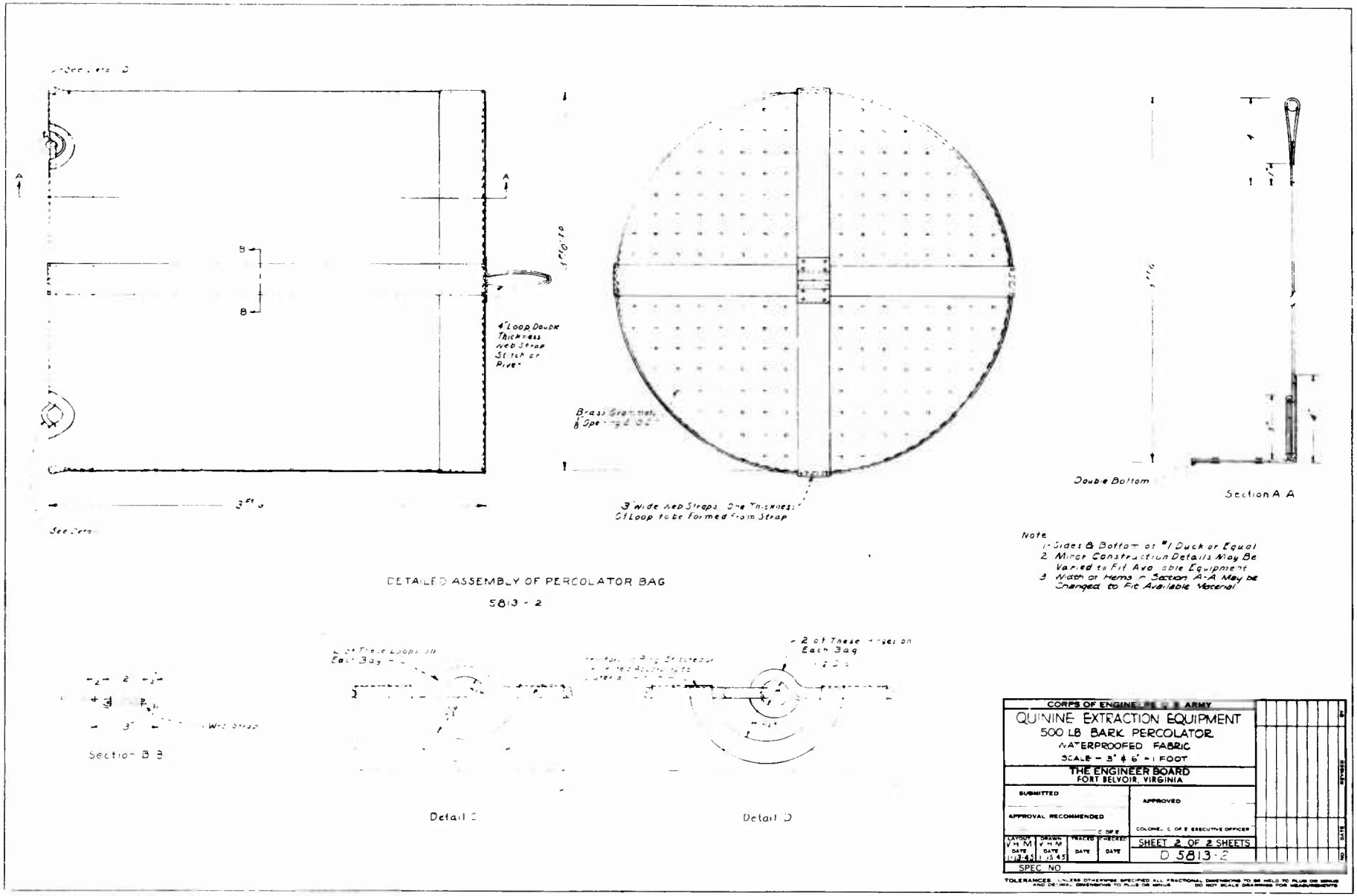
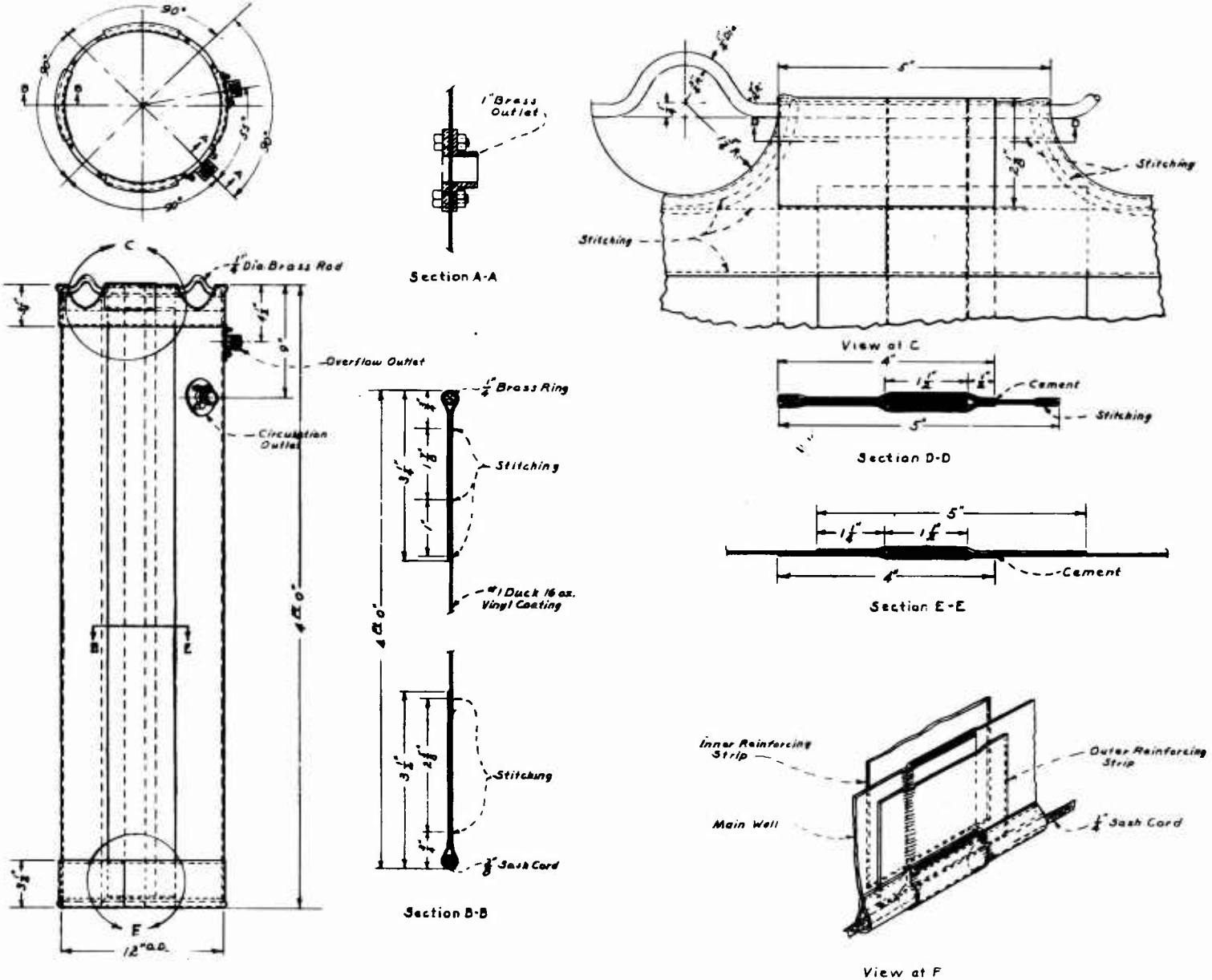
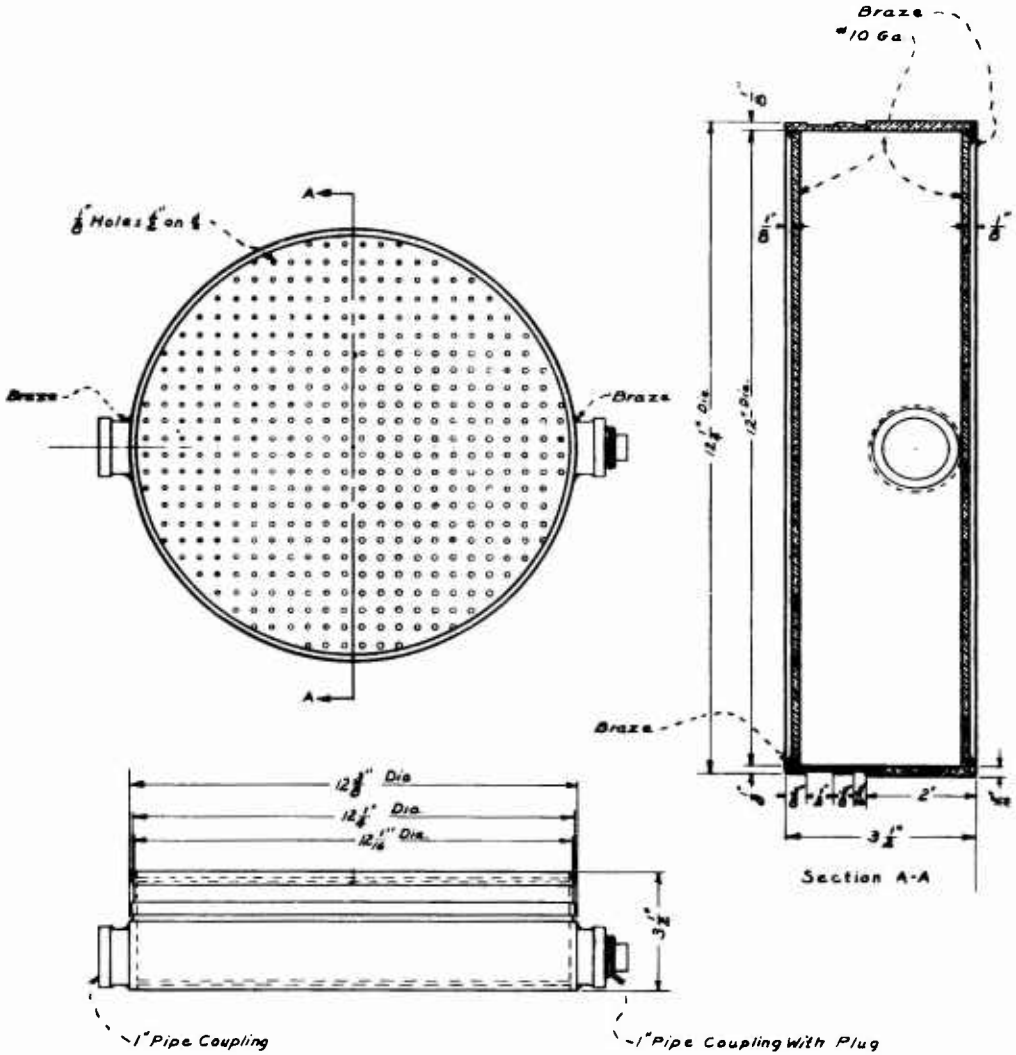


FIG. 30. PLAN, ELEVATION, DETAIL, BARK PERCOLATOR

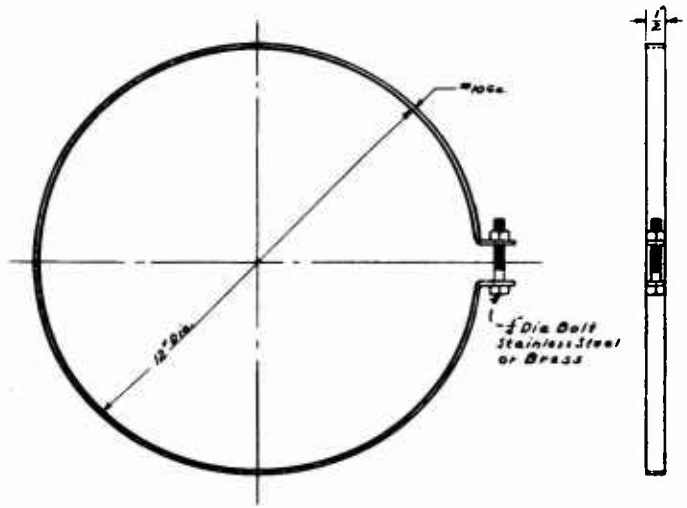


ION EXCHANGE COLUMN
Material as shown
6047-1

FIG. 31. PLAN, ELEVATION, DETAIL, ION EXCHANGE COLUMN



DIFFUSER
Brass
6047-2-1



CLAMPING RING
Brass
6047-2-2

FIG. 32. PLAN, ELEVATION, DETAIL, DIFFUSER

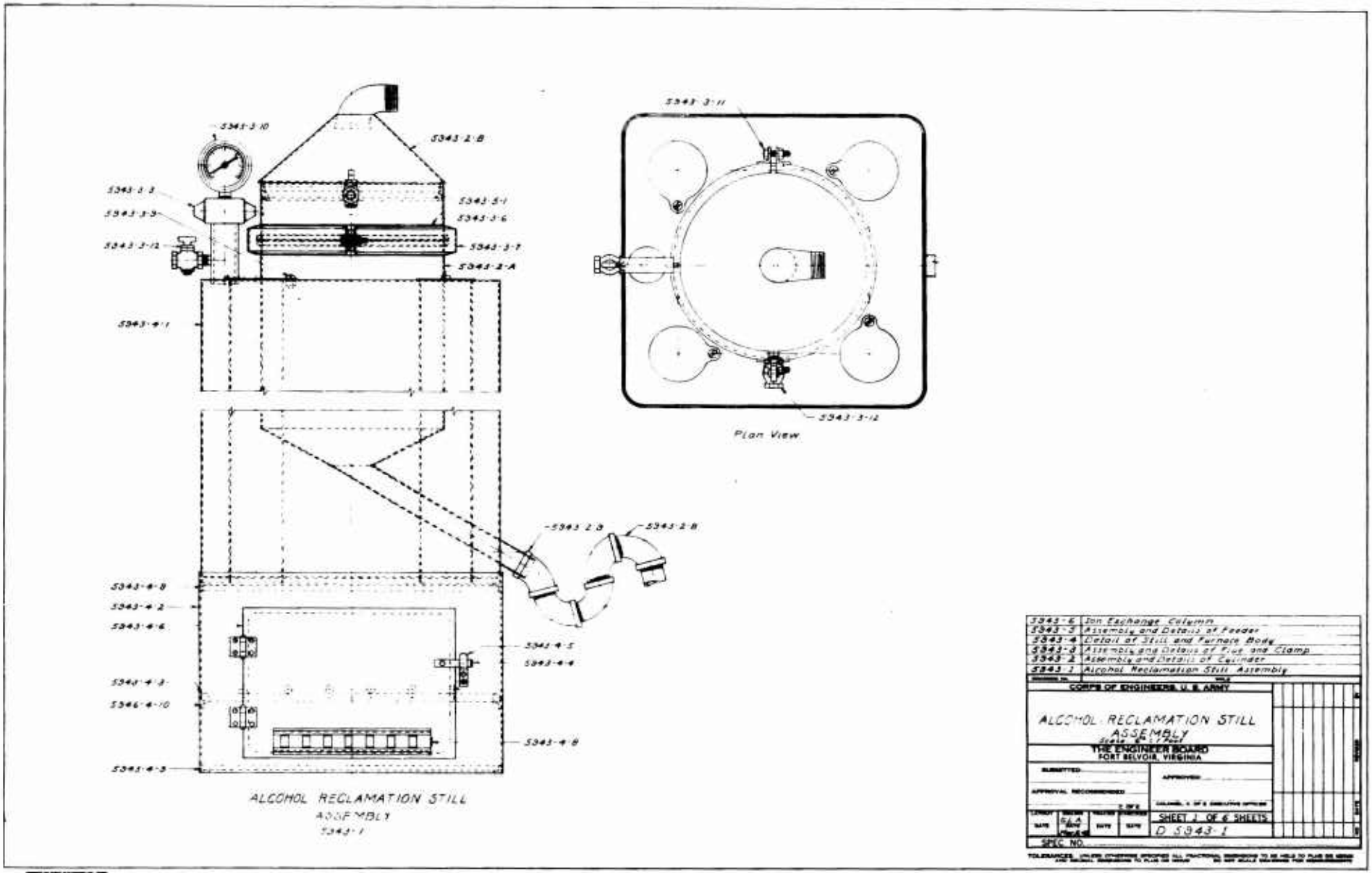
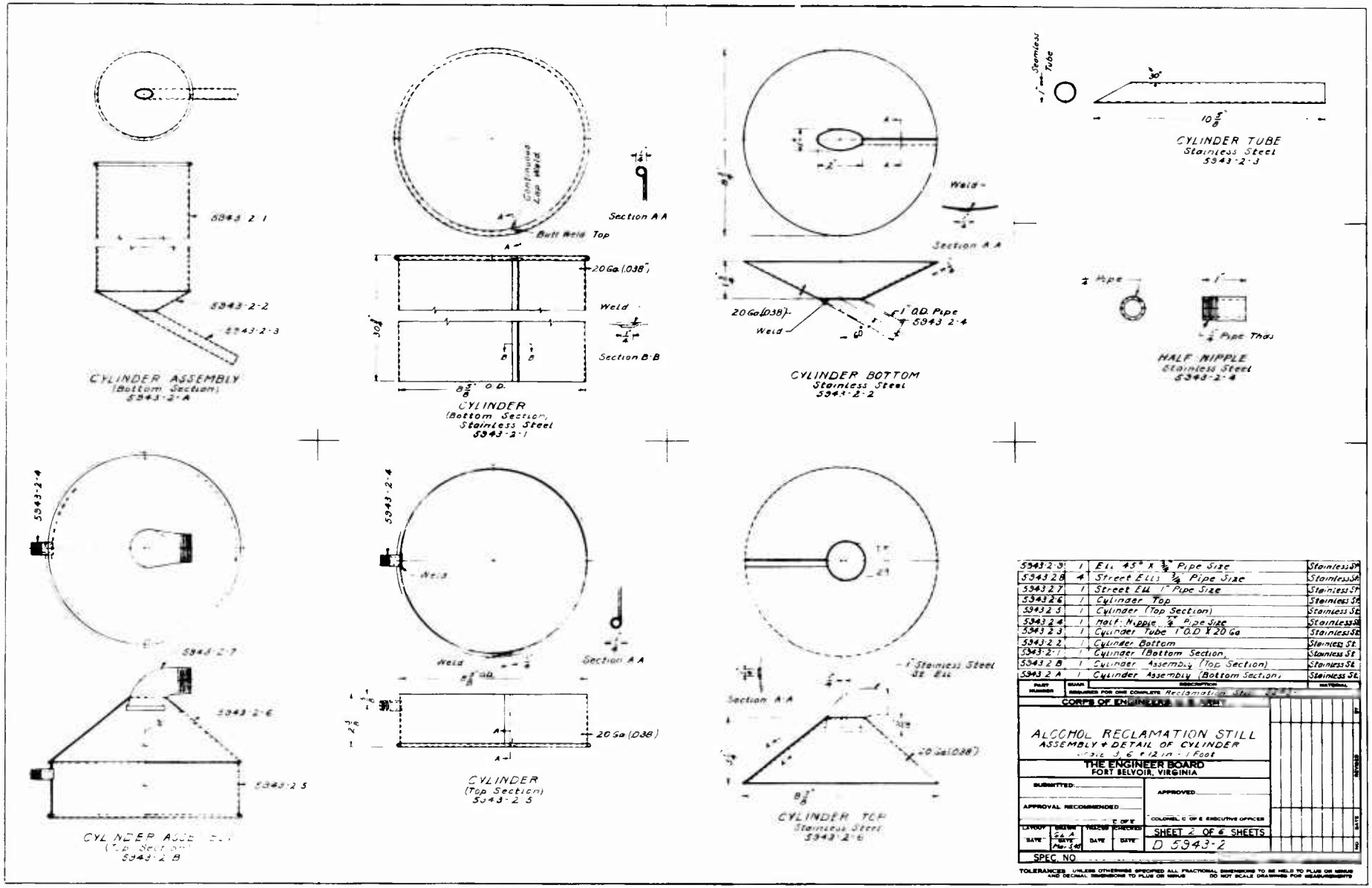


FIG. 33. PLAN, ELEVATION, DETAIL, ALCOHOL RECLAMATION STILL



PART NUMBER	QUAN	DESCRIPTION	MATERIAL
5943-2-9	1	El. 45° x ¾ Pipe Size	Stainless St
5943-2-8	4	Street Ells ¾ Pipe Size	Stainless St
5943-2-7	1	Street Ell 1" Pipe Size	Stainless St
5943-2-6	1	Cylinder Top	Stainless St
5943-2-5	1	Cylinder (Top Section)	Stainless St
5943-2-4	1	Half Nipple ¼ Pipe Size	Stainless St
5943-2-3	1	Cylinder Tube 1 O.D. X 20 Ga	Stainless St
5943-2-2	1	Cylinder Bottom	Stainless St
5943-2-1	1	Cylinder (Bottom Section)	Stainless St
5943-2-B	1	Cylinder Assembly (Top Section)	Stainless St
5943-2-A	1	Cylinder Assembly (Bottom Section)	Stainless St

DRAWINGS FOR ONE COMPLETE Reclamation Still

CORPS OF ENGINEERS

ALCOHOL RECLAMATION STILL
ASSEMBLY & DETAIL OF CYLINDER
SCALE 3" = 12 in = 1 Foot

THE ENGINEER BOARD
FORT BELVOIR, VIRGINIA

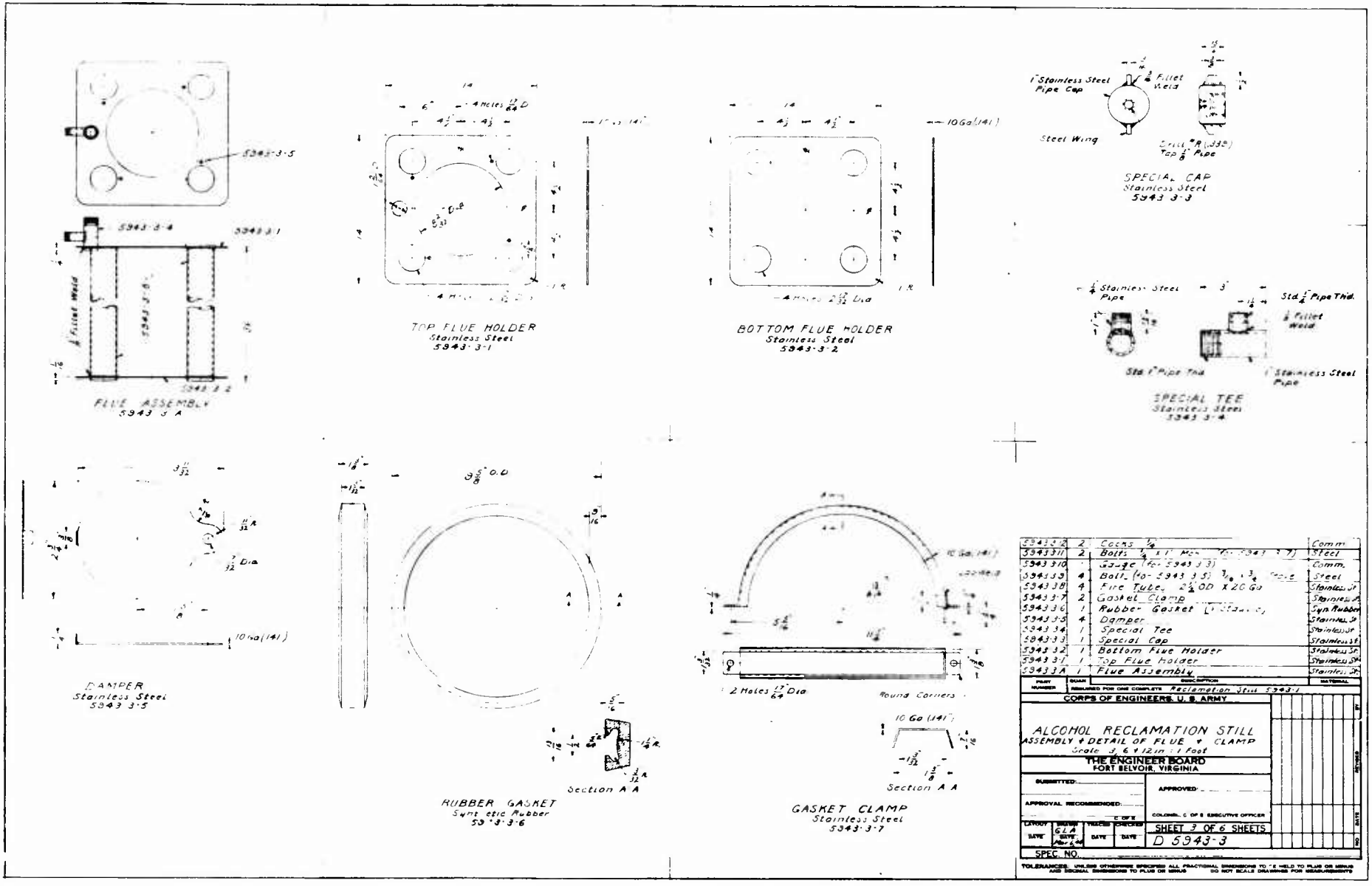
SUBMITTED: APPROVED:
APPROVAL RECOMMENDED:
COLONEL, C OF E EXECUTIVE OFFICER
C OF E

LAYOUT DATE | DRAWN 5-4-45 Rev 5-46 DATE | TRACED DATE | CHECKED DATE

SHEET 2 OF 6 SHEETS
D 5943-2
SPEC NO

TOLERANCES UNLESS OTHERWISE SPECIFIED ALL FRACTIONAL DIMENSIONS TO BE HELD TO PLUS OR MINUS AND DECIMAL DIMENSIONS TO PLUS OR MINUS DO NOT SCALE DRAWINGS FOR MEASUREMENTS

FIG. 34. PLAN, ELEVATION, DETAIL, ALCOHOL RECLAMATION STILL



PART NUMBER	QUAN	DESCRIPTION	MATERIAL
5943-3-8	2	Cocks 1/4	Comm
5943-3-11	2	Bolts 1/4 x 1" Mach (for 5943-3-7)	Steel
5943-3-10	1	Gauge (for 5943-3-3)	Comm.
5943-3-9	4	Bolt (for 5943-3-5) 3/8 x 3/4 Hex	Steel
5943-3-8	4	Fire Tube 2 1/2 OD X 20 Ga	Stainless St
5943-3-7	2	Gasket Clamp	Stainless St
5943-3-6	1	Rubber Gasket (1 Strip)	Syn. Rubber
5943-3-5	4	Damper	Stainless St
5943-3-4	1	Special Tee	Stainless St
5943-3-3	1	Special Cap	Stainless St
5943-3-2	1	Bottom Flue Holder	Stainless St
5943-3-1	1	Top Flue Holder	Stainless St
5943-3A	1	Flue Assembly	Stainless St

REQUIRED FOR ONE COMPLETE Reclamation Still 5943-1

LAYOUT	DRAWN	TRACED	CHECKED
DATE	GLA DATE Apr 44	DATE	DATE

SUBMITTED: APPROVED:
APPROVAL RECOMMENDED: C OF S
COLONEL, C OF S EXECUTIVE OFFICER

TOLERANCES: UNLESS OTHERWISE SPECIFIED ALL FRACTIONAL DIMENSIONS TO 1/8 HELD TO PLUS OR MINUS AND DECIMAL DIMENSIONS TO PLUS OR MINUS .01 DO NOT SCALE DRAWINGS FOR MEASUREMENTS

FIG. 35. PLAN, ELEVATION, DETAIL, ALCOHOL RECLAMATION STILL

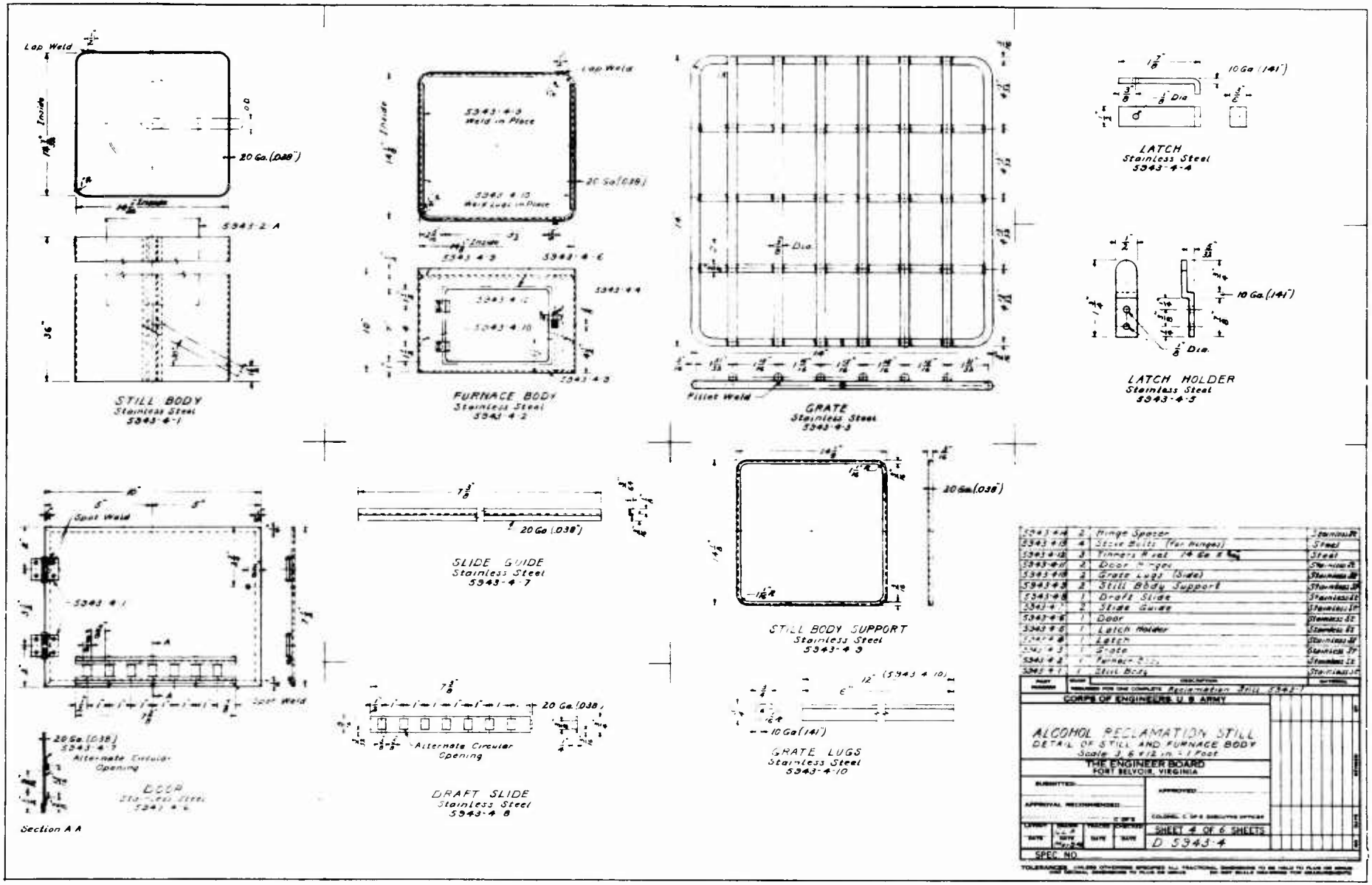
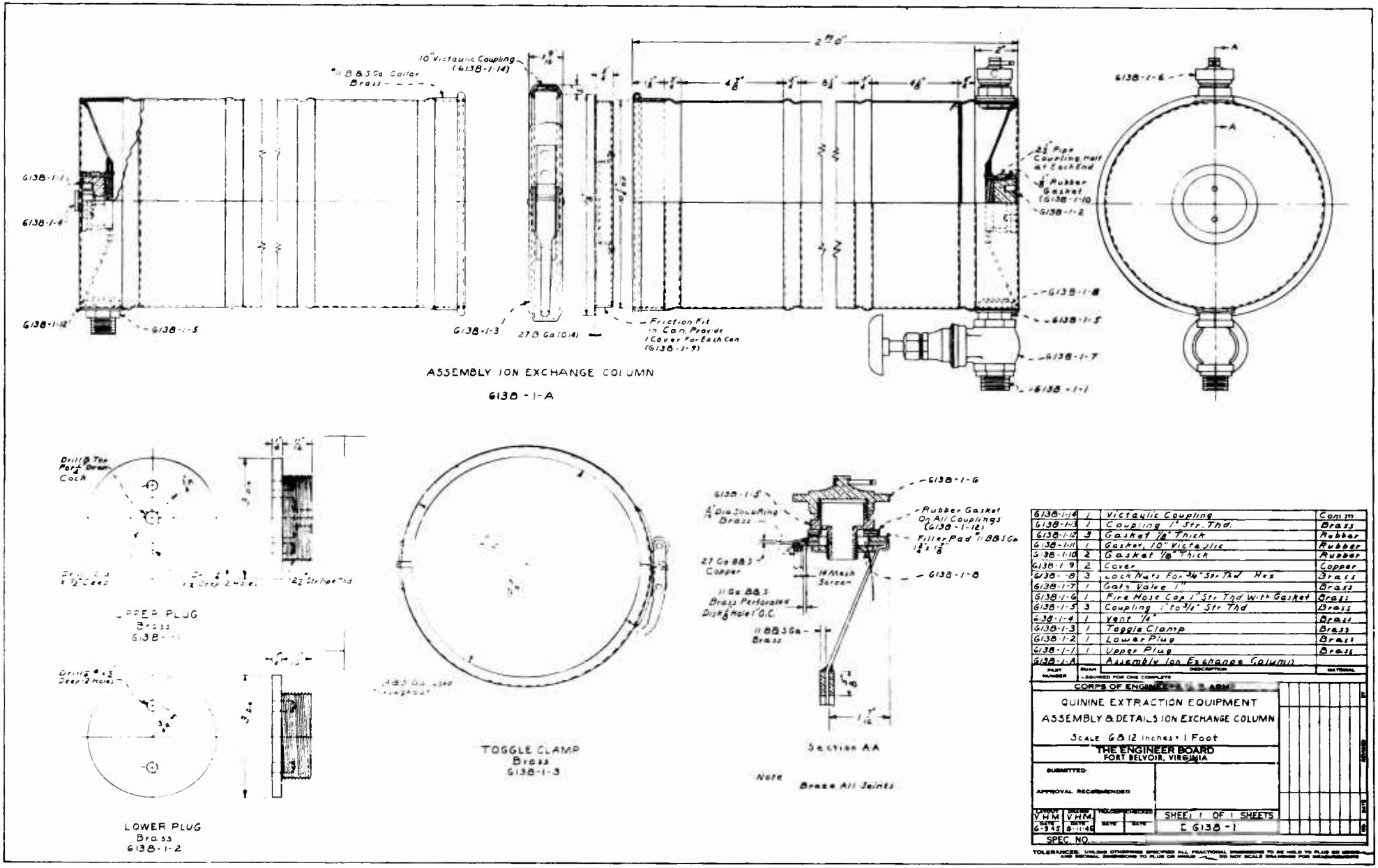


FIG. 36. PLAN, ELEVATION, DETAIL, ALCOHOL RECLAMATION STILL



6138-1-14	1	Victaulic Coupling	Comm
6138-1-13	1	Coupling 1" Str. Thd.	Brass
6138-1-12	3	Gasket 1/8" Thick	Rubber
6138-1-11	1	Gasket, 10" Victaulic	Rubber
6138-1-10	2	Gasket 1/8" Thick	Rubber
6138-1-9	2	Cover	Copper
6138-1-8	3	Lock Nuts For 3/4" Str. Thd Hex	Brass
6138-1-7	1	Gate Valve 1"	Brass
6138-1-6	1	Fire Hose Cap 1" Str Thd With Gasket	Brass
6138-1-5	3	Coupling 1" to 3/4" Str Thd	Brass
6138-1-4	1	Vent 1/4"	Brass
6138-1-3	1	Toggle Clamp	Brass
6138-1-2	1	Lower Plug	Brass
6138-1-1	1	Upper Plug	Brass
6138-1-A		Assembly Ion Exchange Column	

PART NUMBER | EACH REQUIRED FOR ONE COMPLETE | DESCRIPTION | MATERIAL

CORPS OF ENGINEERS, U.S. ARMY

QUININE EXTRACTION EQUIPMENT

ASSEMBLY & DETAILS ION EXCHANGE COLUMN

SCALE 6 & 12 Inches = 1 Foot

THE ENGINEER BOARD
FORT BELVOIR, VIRGINIA

SUBMITTED

APPROVAL RECOMMENDED

LAYOUT	DRAWN	TRACED	CHECKED
V H M	V H M		
DATE 6-3-45	DATE 9-11-45	DATE	DATE

SHEET 1 OF 1 SHEETS

E 6138-1

SPEC. NO.

TOLERANCES: UNLESS OTHERWISE SPECIFIED ALL FRACTIONAL DIMENSIONS TO BE HELD TO PLUS OR MINUS ... AND DECIMAL DIMENSIONS TO PLUS OR MINUS ... DO NOT SCALE DRAWING FOR DIMENSIONS

FIG. 37. ASSEMBLY AND DETAILS, ION EXCHANGE COLUMN